Microsatellite-based characterization of southern African domestic pig (*Sus scrofa domestica)* breeds

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Microsatellite-based characterization of southern African domestic pig (*Sus scrofa domestica)* breeds

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2010

DELARATION

I declare that the _____ (mini-dissertation / dissertation / thesis) hereby submitted to the University of Limpopo, for the degree of _____ (degree & filed of research) has not previously been submitted by me for a degree at this or any other university; that it is my work in design and in execution, and that all material contained herein has been duly acknowledged.

_____ _____

Initials & Surname (Title) **Date**

Student Number: _____

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ABSTRACT

A genetic characterization study was performed on three commercial and three indigenous southern African pig populations. A total of 350 pigs from across southern Africa were genotyped at 40 microsatellite loci. This study represents the first project in South Africa aimed at determining the population structure and genetic diversity of pig populations using microsatellite markers. The specific aims of this study were (i) to optimize and validate a set of microsatellite markers for application in populations; and (ii) to genetically characterize commercial and indigenous pig populations of southern Africa using these markers.

The three commercial pig populations selected for this study were the SA Landrace (SAL), Large White (LAW) and Duroc (DUR). The three indigenous pig populations were the Namibian (NAM), Mozambican (MOZ) and the Kolbroek (KOL). The Kune-kune breed was added as an unrelated reference group.

Hair samples of pigs were collected for DNA extractions. This source of DNA proved to be very successful during the current study. A panel of microsatellite loci developed for the Pig Genome Project was used to genotype animals. The markers used were all polymorphic, with a total of 445 alleles detected. The number of alleles per locus ranged from 3 to 21. A total of 122 rare alleles (with frequencies below 0.05) were observed at 37 of the 40 loci. The expected heterozygosity estimates ranged from 0.531 to 0.692 with an average value of 0.611 across all populations. All loci screened showed deviations from Hardy-Weinberg Equilibrium (HWE) except locus SW1041. A per locus analysis revealed that most loci deviated from HWE due to a heterozygote deficit.

The average rate of inbreeding (*F*is) ranged from 0.082 (commercial populations) to 0.101 (indigenous populations). The low level of inbreeding in the commercial populations could be due to careful breeding strategies. The higher level of inbreeding among the indigenous populations may be explained by homogenous selection or gene flow restriction. The Kune-kune population presented the highest F_{IS} value of 0.253, which suggests a degree of isolation or a small founder population. Screening

based on the Stepwise Mutation Model showed the signature of historic bottlenecks in the SAL, LAW, MOZ and KOL populations.

The overall F_{ST} values among the commercial populations were 0.157, with a value of 0.154 among the indigenous populations. F_{ST} values ranged between 0.112 and 0.270 among the pig populations. Only 17.9% of the total genetic variation could be attributed to differentiation between the populations, suggesting reproductive isolation and low gene flow between populations. The trends observed for F_{ST} were confirmed by R_{ST} values. The genetic distances (D_A) ranged from 0.151- 0.446. The dendrogram based on a neighbour-joining (NJ) method showed that the Kune-kune population groups with the indigenous breeds. The three commercial populations clustered together in a separate group.

Bayesian cluster analysis showed that the most likely of true genetic populations (K) was seven. The results showed that individuals nearly always shared membership coefficients in inferred clusters. In several populations, individuals had partial membership in multiple clusters. The analysis of population structure indicates admixture among breeds.

The results of this study confirm that the indigenous pig populations represent a valuable reservoir of allelic diversity, even though the current levels of inbreeding raise concerns. The valuable genetic structure and phylogenetic information obtained in this study should assist future conservation and population management strategies.

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ABBREVIATIONS

A	Adenosine
An	Average number of alleles
ABI	Applied Biosystems
AD	After Christ
AI	Artificial Insemination
AFLP	Amplified Fragment-Length Polymorphism
AMOVA	Analysis of molecular variance
AnGR	Animal Genetic Resource
ARC	Agricultural Research Council
BC	Before Christ
bp	Base pairs
CBD	Convention on Biological Diversity
cM	Centimorgan
°C	Degrees Celsius
D_A	Modified Cavali-Sforza genetic distance
DAD-IS	Domestic Animal Diversity Information System
DNA	Deoxyribonucleic Acid
dNTP	Deoxynucleotryphosphate
D*s*	Nei´s standard genetic distance
DUR	Duroc
FACT	Farm Animal Conservation Trust
FAO	Food and Agricultural Organization
FAO-ISAG	Food and Agricultural Organization-International Society of Animal Genetics
F_{IS}	Rate of inbreeding
F_{ST}	Standardized variance in allele frequencies among populations
He	Expected heterozygosity
Ho	Observed heterozygosity
HWE	Hardy-Weinberg Equilibrium
IAM	Infinite allele model
k	Number of alleles per locus
K	Number of clusters
KK	Kune-kune
KOL	Kolbroek
LAW	Large White
MCMC	Markov Chain Monte Carlo
MH	Malignant Hypothermia
mM	Millimolar
MNA	Mean number of alleles
MoDAD	Measurement of Domestic Animal Genetic Diversity
M	Molar
MOZ	Mozambique
MS	Microsoft
mtDNA	Mitochondrial Deoxyribonucleic Acid
NAM	Namibia
NDA	National Department of Agriculture
Nem	Gene flow

ng	Nanogram
NJ	Neighbour-Joining method
PCR	Polymerase Chain Reaction
PIC	Polymorphic Information Content
%	Percentage
pMOL	Picomole
QTL	Quantitative Trait Loci
RAPD	Random Amplified Polymorphic DNA
RFLP	Restriction Fragment-Length Polymorphism
Rs	Allelic richness
R_{ST}	Unbiased estimator
PAGE	Polyacrylamide gel electrophoreses
SADC	Southern African Development Community
SAL	SA Landrace
SAPPO	South African Pig Producers Organisation
SMM	Stepwise mutation model
SNP	Single Nucleotide Polymorphism
STR	Simple Tandem Repeat
Taq	*Thermus aquaticus*
TBE	Tris-Boric Acis-EDTA
TEMED	Tetramethylenediamine
U	Units
UPGMA	Unweighted Pair-Group Method of Averages
UK	United Kingdom
USA	United States of America
VNTR	Variable Number of Tandem Repeats
v/v	Volume per volume
µl	Microlitre
µM	Micromole

CHAPTER 1

Introduction

1.1 General introduction to the species

Seventeen species of pigs and hogs in eight genera make up the modern family *Suidae* (Figure 1.1). Pigs are even-toed ungulates belonging to the order Artiodactyla (Ruvinsky and Rothschild, 1998). These medium-sized animals are typically stocky with a barrel-like body. The skin is usually thick and sparsely haired. Body length ranges from 58-190 cm with a weight of up to 275 kg. Pigs are omnivores and have a two-chambered stomach and do not ruminate (Chen *et al.*, 2007).

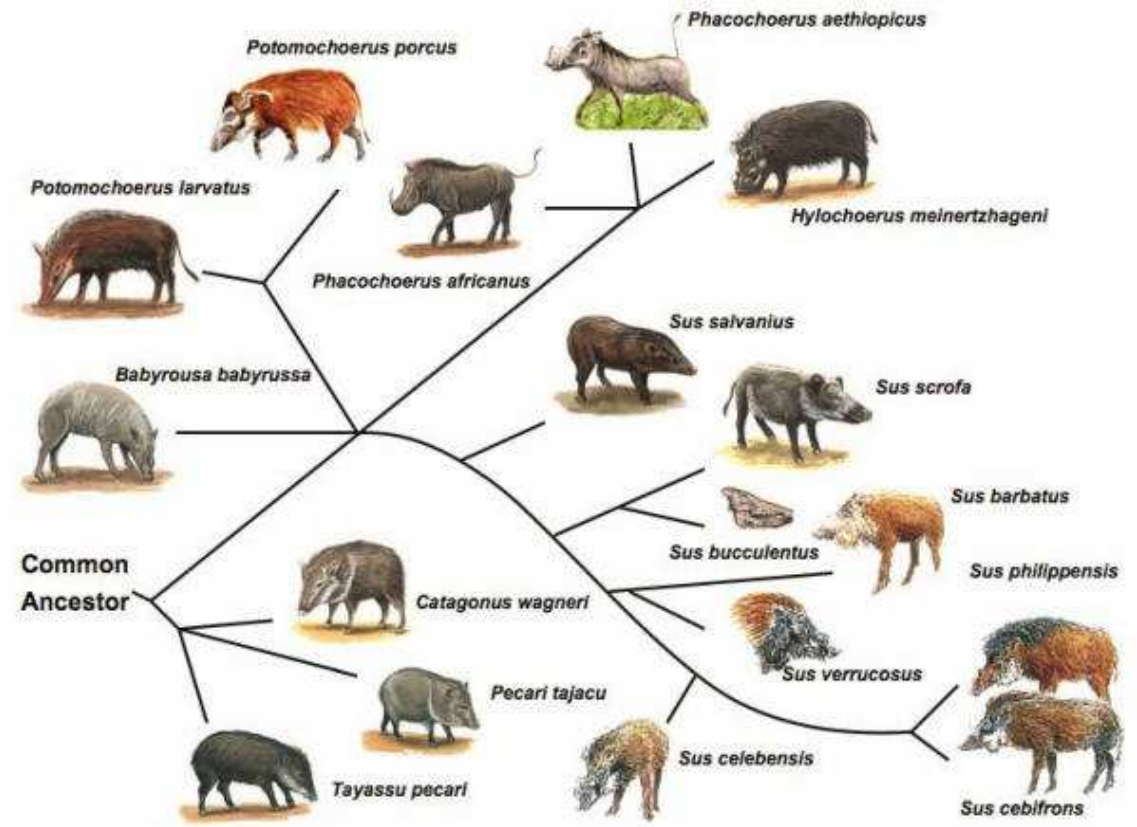

Figure 1.1 Family *Suidae.* Source: Randi *et al.* (1996); Groves *et al.* (1997); Fokkinga (2004); Robins *et al.* (2006)

Chromosome number and chromosome morphology are basic to a thorough understanding of the genetics of an organism. In the first recorded investigation of pig chromosomes (Poland China boar), a diploid number of 16 in males and 18 in females, was reported (Wodsedalek, 1913). The normal karyotype of domestic pig (*Sus scrofa domestica*) contain 2n = 38 chromosomes as seen in Figure 1.2 (a) and (b). The karyotypes of both domestic pig and wild boar (*Sus scrofa scrofa*) are very similar (Bosma, 1976; Rejduch *et al*., 2003).

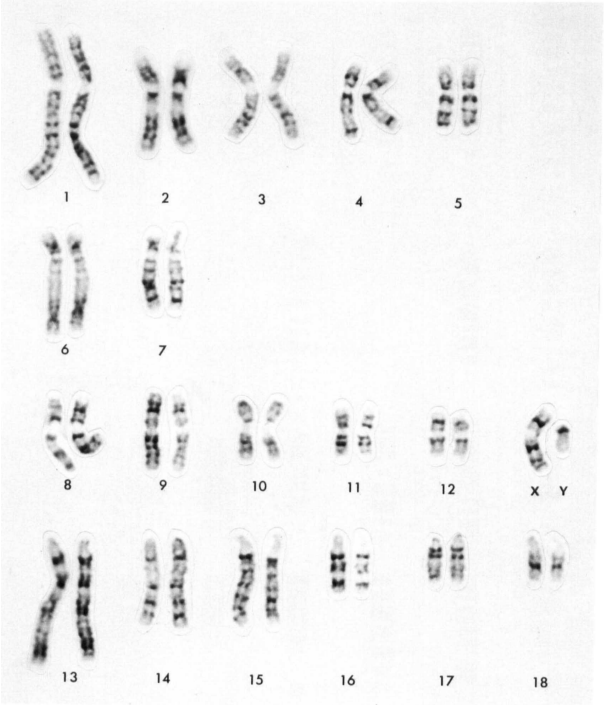

Figure 1.2 (a) Representative GTG-banded male domestic pig karyotype and ideogram (Source: Gustavsson *et al*., 1972)

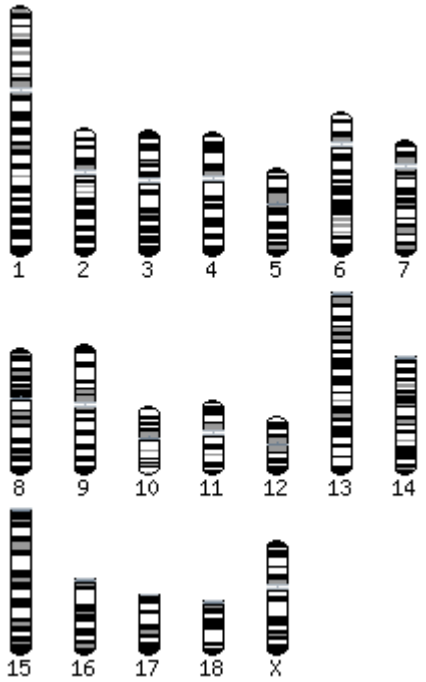

Figure 1.2 (b) Ideogram of a male pig (Source: Gustavsson *et al*., 1972)

Pigs originally occurred across Eurasia and throughout Africa (Ruvinsky and Rothschild, 1998). Humans used the Eurasian wild boar (*Sus scrofa*) to develop domesticated pigs (*S. scrofa domestica*). Domestication is the process of genetically adapting a wild biological organism to better suit the needs of human beings, as a result of living and breeding conditions under careful human control for multiple generations (Darwin, 1868).

Pig fossils are known to be from the Oligocene of Europe and Asia and the Miocene of Africa (Oliver and Brisbin, 1993; Scherf, 1995). Domestication of pigs seems to have taken place outside Africa and they were introduced, rather than domesticated (Plug and Badenhorst, 2001) into southern Africa. According to archaeologists, South Africa was occupied solely by San hunter-gatherers before the time of Christ. These people survived by hunting rather than keeping domesticated livestock. Domesticated animals are thought to have originated in the Middle East about 9,000 years ago (Giuffra *et al*., 2000). The coming of Islam to North and East Africa seemed to have limited the migration of pigs into southern Africa – consequently pig remains are not common in southern African excavation sites (Epstein and Masen, 1971a); Plug and

de Wet, 1994). This does not mean that domestic pigs were completely absent, but it does indicate that they were not generally kept (Plug, 1996).

While most livestock were utilized initially by nomadic people, pigs are more indicative of a settled farming community (Briggs, 1983). Relative to cattle, sheep and goats, pigs played an insignificant role as livestock of the early pasturalists in southern Africa. The unsuitability for a nomadic lifestyle, religious taboos, diseases and the tropical nature of large regions all favoured alternative types of livestock (Bonsma and Joubert, 1957; Plug, 1993; Clutton-Brock, 1997; Bester and Küsel, 1998).

Personal communications with Dr. Ina Plug, an archaeologist from the Transvaal National Museum and Mrs Jenny Bester, from the Agricultural Research Council (ARC), confirmed that there is very little historical information available regarding the southern African indigenous pig populations. There were apparently three phases of migration and introduction of domesticated animals into Africa, central Africa and southern Africa. The process of barter, warfare and migration resulted in a southern movement of animals down the length of Africa. Archaeological finds suggested that a further southward migration took place in southern Africa as early as 400 BC but, certainly, by 200 AD the Khoi-Khoi pasturalists arrived at South Africa's northern borders with early sheep populations. A second phase of migration between the 3rd and 7th centuries brought Iron Age communities into the eastern parts of the country with cattle, sheep, goats, chickens and only one archaeological record of pig introduction (Clutton-Brock, 1997; Plug and Badenhorst, 2001). The last phase of introduction began in the 16th to 17th century when the Dutch landed in the Cape to establish a halfway station on the sea route to the East and the European pig populations were introduced (Bester and Küsel, 1998).

Pigs were also introduced to South Africa from the island of St Helena at the end of 1685. Nevertheless, only 24 pigs were on the livestock inventory list (SA Studbook and Livestock Improvement Association, available at *http://www.studbook.co.za*) with the establishment of the SA Studbook association in 1905. In the 1880's pigs were recorded in Pondoland, Tongaland, Lesotho and Hereroland (Mason and Maule, 1960; Epstein and Mason, 1971b).

Chinese and Portuguese trading ships passed South African shores (Ramsay *et. al.*, 1994) and pigs were most likely exchanged with the indigenous communities (Quin, 1959). All other archaeological records on pigs from the sub-region date to post-European contact (Plug and Badenhorst, 2001).

Domestication of livestock occurred thousands of years ago and the development of specialized breeds probably dates back many centuries. Many of these breeds, which included horses, donkeys and pigs, have adapted to local conditions and have acquired unique characteristics. They are heat tolerant, hardy and fairly disease resistant (Plug and de Wet, 1994).

The existing genetic composition of existing livestock populations is the result of previous selection and is not always the best for the population (Maree, 1994). Interactions between environmental and human selection have led to the development of genetically distinct populations.

Pig farming in different environmental conditions has resulted in populations with traits such as heat/cold tolerance and disease resistance, which favour their survival under environmental stresses (Maree, 1994). Farmers have also selected for a variety of attributes with a major focus on productive traits such as meat yields and fertility.

1.2 The South African Pig Industry

The pig industry of South Africa has grown over the past 345 years to a dynamic industry. The following indicate the status of the infrastructure of the commercial pig industry with reference to stud animals and Pig Breeder's Societies:

A national breeding herd consisting of approximately

- 46 registered pig abattoirs responsible for the slaughtering of 86.5% of the 1.6 million pigs in 2007 (Table 1.1).
- 28 active members and breeding companies affiliated to the Pig Breeder's Society (PBS) of South Africa with 9,216 registered females and 1,385 registered boars in 2007/2008 (Table 1.2).

- 103,385 females and 7,000 males owned by approximately 700 pig farmers (males not shown) (personal communications, Mr F. Voordewind, 2009) (Table 1.3).
- 210 active members affiliated to the South African Pig Producers Organisation (SAPPO) with 93,733 registered sows and an average herd size of 421 per province (Table 1.4).

Table 1.1 Provincial statistics: distribution of farm animals in the RSA (thousand) (2007)

Province	Cattle	Sheep	Goats	Pigs	Poultry	Ostriches
	Thousands					
Eastern Cape	3,182	7,313	2,650	**106**	1,518	113.9
Free State	2,323	4,891	255	**123**	3,540	5.72
Gauteng	270	90	42	**181**	4,044	4.45
KwaZulu-Natal	2,934	809	931	**156**	12,804	N/A
Limpopo	1,026	204	1,118	**416**	825	0.3
Mpumalanga	1,411	1,703	96	**128**	10,300	0
Northern Cape	484	6,221	554	**29**	238	13
North West	1,769	639	798	**331**	16,585	13
Western Cape	532	2,639	234	**181**	12,345	226.4
Total	13,934	24,511	6,682	**1,654**	61,986	376.7

Table 1.2 Breed/breeder activities in the national pig performance and progeny testing scheme (2008)

Breed	Number registered stud animals and involved in scheme
SA Landrace	
Sows	1,569
Boars	385
Breeders	8
Duroc	
Sows	894
Boars	201
Breeders	5
Large White	
Sows	6,753
Boars	799
Breeders	15
Total	
Sows	**9,216**
Boars	**1,385**

Table 1.3 Total **c**ommercial sows (males not shown) in South African Provinces (2009)

Province	**Commercial Sows**
Gauteng	11,300
Limpopo	11,700
Mpumalanga	14,000
North West	17,800
Kwa Zulu Natal	16,600
Western Cape	16,385
Free State	9,000
Northern Cape	2,000
Total	**103,385**

Table 1.4 South African Pig Producers Organisation, membership, registered sows and average herd size/province (2009)

Province	**Pigs/province (%)**	**Number active members**	**No. of registered sows**	**Average herd size/province**
Eastern Cape	4.9	1	4,600	4,600
Free State	9.6	34	9,000	264
KwaZulu-Natal	17.7	70	16,600	237
Northern Provinces*	48.2	72	45,148	627
Western Cape	17.5	44	16,385	372
Northern Cape	21.1	2	2,000	1,000
TOTAL	**100**	**210**	**93,733**	**421**

*Gauteng, Limpopo, Mpumalanga and North West

The pig industry in South Africa is essentially an intensive industry with indoor housing. The pig is the most efficient meat producing farm animal and, next to the dairy cow, the most efficient converter of cereals and their by-products into an edible product containing animal protein (Taverner and Dunkin, 1996). Profitable pig production depends on a high fertility level, a fast growth rate, a high percentage of lean meat carcasses, health aspects, size of the national herd and percentage animals suitable for slaughtering. This can only be achieved with proper management and feeding and especially selected genetic material (Visser, 2004a).

Breeding programs include pure breeding, linebreeding, outbreeding, grading-up and crossbreeding (Visser *et al*., 1993)**.** The demands posed by artificial selection could only be satisfied with the importation of suitable pig breeds into South Africa. Indigenous pigs were unsuitable even as basic breeding stock, due to their slow growth and inadequate meat production, therefore indigenous pigs play no role in intensified commercial units (Maree, 1994). This is still the case, as the pig industry is very competitive comprising an intensive breeding system that is based on producing the best products in a short period of time (Visser, 2004a and Prolit, 2004).

The Pig Breeder's Society of South Africa was formed in 1919 and has been affiliated since its establishment with the South African Studbook and Livestock Improvement Association. The first importation of live stud pigs into South Africa took place in the early 1920's. Thereafter gradual importations of live animals and semen took place mainly from England, the Netherlands, Canada, the USA, Sweden and Germany (SA Studbook and Livestock Improvement Association, available at *http://www.studbook.co.za*). During the last decade only frozen semen imports have been permitted and only from approved genetic resources (according to international regulations controlled by the Pig Breeder's Societies), to maintain the health status of local pig populations.

Government Institutions such as the establishments at Irene, Cedara and Potchefstroom and the Experimental Farm of the University of Pretoria, did a great deal to improve the genetic standard of South African pigs. The South African Pig Improvement Scheme was established in April 1956. The success of this scheme can be attributed to a dedicated team effort between the former Meat Board, the Pig Breeders Society, the Department of Agriculture, the ARC and the Industry (Department of Agriculture, 2006).

The following are registered pig breeds with the SA Studbook and Livestock Improvement Scheme: SA Large White, Chester White, Piétrain, SA Landrace, Hampshire, QM Hamline, Duroc and the Large Black (Campher *et al*., 1998). The predominant pig breeds are the South African Landrace, the Large White, the Duroc and the Piétrain (Visser *et al*., 1993).

There are two recognised indigenous populations, namely the short-snouted, pot-bellied 'Kolbroek' and the long-snouted "Windsnyer". The South African hard-footed or 'hut' pigs are found free-ranging in and around rural areas where they are often used as foragers/scavengers and converters of otherwise unutilized kitchen and garden refuse (Emmett, 2004). Their proximity to villages has lead to the general term and has the perception of not having real value from a western perspective. Progressive trends towards more environmentally effective pig production and small-farm systems – along with data on the potential of the 'hut' pig as converters of fibre, root and leaf

crops and as links in integrated small farm systems, have however shown that these animals could become valuable livestock (Ramsay *et al*., 1994) to the small-scale rural farmer.

Registered pigs make up only a small proportion of the national herd (Table 1.1). The rest of the population consists of unregistered animals and crossbreds. Local pig producers must perform consistently in order to survive in a very competitive market. Although the South African pig industry is relatively small it is able to meet the local demand. Furthermore, South Africa has exported many pigs to countries such as the Congo, Zimbabwe, Zambia, Malawi, Zambia, Reunion, Madagascar and the Seychelles. It is an industry that can be expanded rapidly should the demand increase and it requires limited land resources (Maree, 1994).

In the pig meat industry, three general types of commercial pigs are recognized: the lard, meat, and bacon types. Lard-type pigs tend to have a high proportion of body fat and are compactly built. Meat-type pigs are intermediate between the lard and bacon types and combine muscle and body length with the ability to reach a marketable weight without accumulating excessive fat. Bacon-type pigs are common with enterprises where pigs are fed on commercial well formulated rations (SA Studbook and Livestock Improvement Association, available at *http://www.studbook.co.za)*. A description of the major commercial pig populations with reference to their traits, characteristics, qualities and role in the industry follows.

1.3 Commercial populations

There are three main commercial pig populations in South Africa, namely the SA Landrace, Large White and the Duroc. The development and distinct characteristics of each population is discussed below.

1.3.1 The South African Landrace (SAL)

The Landrace breed was originally developed in Denmark in 1895 by first crossing the native Danish pig with the Large White (SA Studbook and Livestock Improvement Association, available at *http://www.studbook.co.za)*. The result was then improved upon during years of selection and breeding under strict government

control. The Danish national pig breeding scheme was established in 1896. Denmark refused to export live pigs until World War II, when representative specimens of the breed were exported to Sweden. Danish farmers had concentrated on producing a pig that suited the British bacon trade, which preferred the 'Wiltshire' type of bacon. The progeny from these pigs eventually reached England and Ireland. The Landrace was also bred to be adaptable to the intensive-housing system of production (Kirsop, 1997).

The first Landrace pigs were imported into South Africa in 1952 from Holland. The Dutch Landrace was a more robust type of animal, whilst the Swedish Landrace was more feminine, that produced a docile pig breed with excellent mothering skills (Briggs, 1983). Through breeding and genetic improvement programmes, local herds made such remarkable progress that the breed became known as the SA Landrace and resulted in the breed gaining entry into a common herd-book register. It is the second most important pure commercial breed in the country (Visser *et al*., 1993).

The SA Landrace has white hair and a pink skin (Figure 1.3). These pigs have lopped ears and the body has a long middle, light forequarters, and excellent ham development. The major faults with the original Landrace were leg weakness, splay legs and nervous disorders such as Porcine Stress Syndrome (PSS). PSS still occurs in some strains.

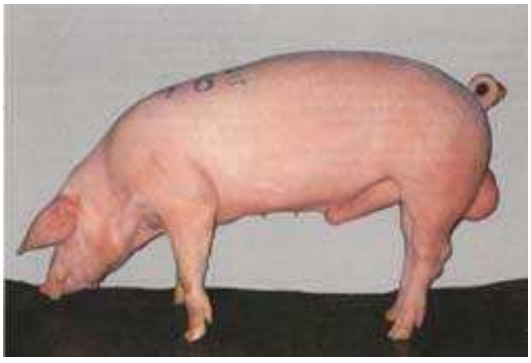

Figure 1.3 Representation of a typical SA Landrace boar

The main breed standards of the SA Landrace include:

- it is noted for its early, rapid growth; its weight at weaning is higher than that of other populations (Table 1.5 and Table 1.6),
- it is not usually as prolific a breeder as the Large White and tends to be slightly fatter (Table 1.5 and Table 1.6),
- feed conversion is inferior to Large Whites (Table 1.5) and
- the SA Landrace undoubtedly has the best mothering traits of all the South African pig populations because it is docile as a pure breed and can be handled easily. Certain strains within the SA Landrace are renowned for extremely good muscularity - hams are well developed, broad and deep (Visser *et al.*, 1993 and Kirsop, 1997).

Table1.5 South African pig registration performance testing Phase D (2009)

Breed	Sex	Number	Age	Fcr	Fat	Tdg	DonT	E Mass
DUROC	Female	606	141.9	2.34	12.1	9245.1	67.5	89.9
DUROC	Male	623	139.5	2.18	9.71	966.9	65.5	90.3
LW	Female	2427	141.3	2.33	11.9	932.6	67	90.7
LW	Male	2556	139.2	2.18	9.4	970.5	62.5	89.1
SAL	Female	724	141.7	2.35	11.9	888.3	69.5	89.7
SAL	Male	537	138.5	2.12	10.6	954.2	66	90

Table 1.6 South African pig registration performance testing (litters born) Phase A (2009)

Breed	Litter	Size	Birth kg	Days	Litter kg	Wean	Days	Litter kg
Duroc	420	9.0	11.5	21.0	35.8	6.4	30.6	46.6
LW	3732	10.9	16.7	20.8	54.5	9.8	29.6	73.3
SAL	926	10.9	17.5	20.3	55.8	9.8	30.7	79.2

1.3.2 The Large White (LAW)

The Large White (Figure 1.4) was first recognized as a distinct breed in England in 1868 and the first herd book was published in1884. During the early 20th century, the Large Whites were exported from England to many other countries across the world. The popularity of the breed has continued to increase around the world, and it is clearly one of the two major maternal populations in the world (Jones, 1998). It is believed that the first significant imported consignment, from abroad, took place after the South African Boer War (1899-1902), when dedicated efforts were made to build up the depleted local pig herds.

The South African Large White has white hair and a pink skin. The body is somewhat sturdier than that of the SA Landrace, it has a good length and is well balanced. The head is characteristic of the breed – the snout is short and fairly dished and the ears are always erect. Any deviation from these two unique characteristics is indicative of impurity of the breed (Visser *et al*., 1993).

Figure 1.4 Representation of a typical Large White sow

The main breed standard of the Large White include:

- the excellent mothering and rearing abilities of female animals combined with general docility (Table 1.5 and Table 1.6),
- performance figures (Table 1.5 and Table 1.6) tend to be better than that of the other populations (Visser *et al*., 1993) and
- the breed is less stress susceptible (Visser *et al*., 1993).

1.3.3 The Duroc (DUR)

During the last quarter of a century, the Duroc (Figure 1.5) has been the fastest growing breed in the world. It was developed in the United States of America (USA), and for many years, all the growth and development of the breed occurred in the USA. It has become one of the most important terminal sires in Canada, Denmark, Japan, China, Taiwan and many other countries around the world (Jones, 1998).

The first Duroc pigs were imported into South Africa from Canada in 1980. The purpose of this importation was to make available a third breed, primarily for cross-breeding purposes. A distinct characteristic is the rusty red colour of the breed although shades can vary from light to dark. The Duroc is renowned for strong bone development.

The Duroc has a rusty red, brown colouring. The head is of medium size. The nose is of medium size and length and the ears point forward and downward, but does not cover the eyes.

Figure 1.5 Representation of a typical Duroc boar

Duroc sows cannot be regarded as good mothers, although they give birth to large litters. However, individual sows within the breed can exhibit good mothering skills.

An advantage of this breed is the fact that stress susceptibility is negligible and terminal offspring can therefore be transported and marketed without any problems. Duroc meat is well marbled - significantly better than that of other populations. Due to the relatively small size of the Duroc gene pool in the country, semen importation from abroad is imperative to continually improve the genetic potential of the breed (Visser *et al*., 1993).

1.4 Indigenous populations

Holness and Smith (1973a and b) regarded the bush pig *(Potamachoerus porcus)* and warthog *(Phacochorus aethiopicus)* to be probably the only truly indigenous members of the family *Suidae*. The livestock of South Africa are not truly indigenous as they originated in the Middle East where domestication of animals began almost 9,000 years ago and were either bartered for or introduced by traders or settlers (Bester *et al.*, 2006). According to FACT (Farm Animal Conservation Trust), there are only two recognized indigenous pig populations in South Africa, namely the Kolbroek and the "Windsnyer". A description of the two populations follows.

1.4.1 The Kolbroek (KOL)

This population resembles a breed of pig common in China. There is evidence that a sailing ship, belonging to the Dutch East India Company was wrecked off the coast at Cape Hangklip and that the pigs on board fell into the hands of farmers who had settled in the area. The name of this ship was the Colebrook. Another explanation of the name is the stripes (breech markings) on the animal may have been another origin of the name Kolbroek. The origin of both the pig and its name is therefore in still unclear.

The Kolbroek is extremely hardy and survives by scavenging outside huts or homesteads. This makes the pig ideal for rural areas where intensive farming is not possible. The first scientific articles on these pigs were written in 1925. In 1932, the Kolbroek was registered in the local agricultural show in Worcester (Agricultural Research Council, available at *http://www.arc.agric.za*).

Kolbroek pigs (Figure 1.6) are very short with pricked ears and a squashed face. The breed is dark coloured being either black or brown and are often striped at birth. Kolbroek pigs thrive on a high fibre diet compared to the Landrace and Large White and can also utilize kikuyu grazing. They have high disease resistance and have a docile nature (Visser, 2004b).

Figure 1.6 Representation of a typical Kolbroek sow

1.4.2 "Windsnyer"

Although it is maintained that all pig populations were introduced by Europeans, it can be seen that the "Windsnyer" (Figure 1.7) quite closely resembles the description

of the ancient Egyptian breed which are small and have bristles forming a distinct mane. Like many of the indigenous animals these pigs have a large colour variation being, either black, reddish-brown, brown, black and white or spotted. Some of the young have longitudinal stripes which are typical of the young bushpig. The name "Windsnyer" (wind-cutter) is derived from its shape as it is narrow-bodied, long-nosed and razor-backed (Agricultural Research Council, available at *http://www.arc.agric.za*).

Figure 1.7 Representation of a typical "Windsnyer" sow

This pig is very hardy and scavenges for its food. It can convert food with a low nutrient content very efficiently, enabling it to survive on food such as the cereal by-products of brewing. It has been shown that the 570 kg food needed by one pig of an imported breed to produce a litter of ten piglets, is sufficient for two and a half indigenous sows and a combined litter of 20 piglets. The "Windsnyer" is also able to survive periods of food shortage. Females of this breed display strong maternal instincts which results in very few piglet deaths. These pigs, however, are very rare and can probably only be found in rural areas scavenging among huts for food. For this reason sampling of this pig breed was not included in this study (Agricultural Research Council, available at *http://www.arc.agric.za*).

1.5 Southern African indigenous pig populations

Other indigenous pig populations exist in southern Africa that is endemic to the region. A short description of these pig populations follows.

1.5.1 Namibian indigenous pig population (NAM)

The pig industry in Namibia is very small and the pig population consists mostly of indigenous pigs. The origin of these pigs are unsure, possibly from areas of the Mediterranean Sea, brought to southern Africa by passing ships and traded with local communities. Adaptability to harsh environments, good fertility, low maintenance requirements, tasty meat, stress tolerant and excellent lard producers are the main qualities of these pigs. These traits were arrived at through natural selection, since the indigenous pigs were never subjected to deliberate selection strategies. Individuals are smaller than pigs from commercial pig populations and their meat is darker and subjectively tastier (Els, 2000).

Namibian indigenous pigs are characterized by a long snout and relatively long and lean body (Figure 1.8). Body colour is mostly mottled brown, black and white, but uniform colours also occur (Figures 1.9 and 1.10). They are mostly found in the northern communal areas of Namibia in and around Rundu in the Kavango region (Els, 2000).

Figure 1.8 Representation of a typical Namibian indigenous pig with long snout and relatively long and lean body

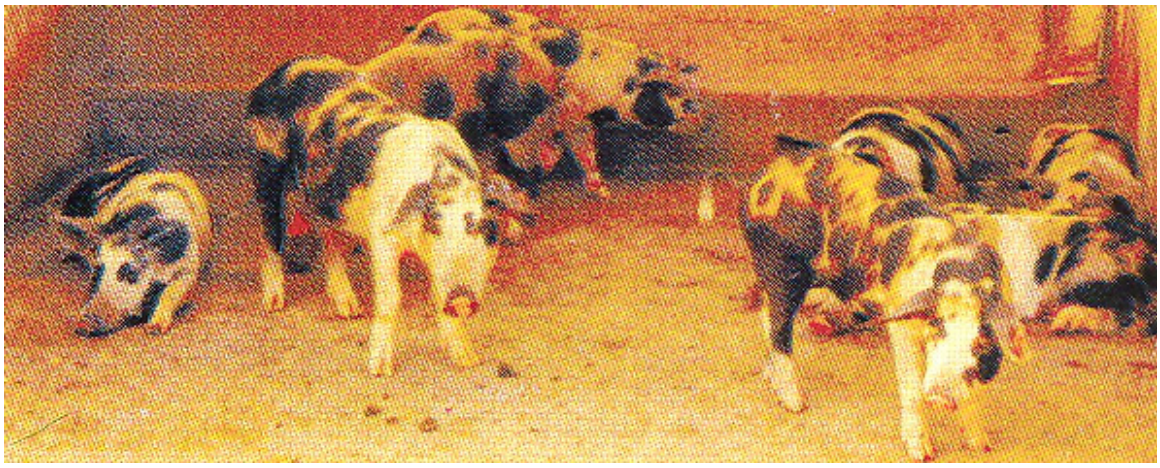

Figure 1.9 Namibian indigenous pigs in a paddock

Figure 1.10 Representation of a typical Namibian indigenous pig with black and white body colouring

1.5.2 Mozambican indigenous pig population (MOZ)

Most of the pigs were found around villages in the Angonia district of the Tete Province, Mozambique, by Dr M-L Penrith, from Onderstepoort Veterinary Institute (Penrith *et al*., 2004). These pigs are of an unimproved type, black-skinned with long hair, long narrow snout and usually have a well developed mane (Figure 1.11). A small percentage of the pigs are not black, but have light coloured hair with black spots. A significant number of the pigs have white feet (Figure 1.12), and a smaller

number have, in addition to white feet, a white belt of rather irregular shape and width over the back. The belly might be quite extensively white. They are sturdy well built pigs (Figure 1.13) that are somewhat larger than the pigs in the rest of Mozambique, which are almost invariably plain black. The ears vary from upright to directed forwards over the eyes. Their eyes, incidentally, vary from brown to quite light colours. There is also variation in the degree to which the mane is developed (Penrith *et al.*, 2004).

Figure 1.11 Representation of a typical Mozambican black-skinned pig with long hair, long narrow snout and a well developed mane

Figure 1.12 A Mozambican piglet with white feet

Figure 1.13 Mozambican pigs are sturdy and well built and are found living around villages

1.6 Reference Group

For this study, Kune-kune pigs were selected as the reference group because of their resemblance to the Kolbroek and Namibian pig populations.

1.6.1 Kune-kune (KK)

Eighteen animals were collected in the late 1970's by Staglands Wildlife Reserve and Willowbank Wildlife Reserve (New Zealand) and this formed the basis of a captive breeding programme (New Zealand Kune-kune Breeder's Association, available at *http://www.kune-kune.co.nz*). Now widely spread throughout New Zealand, with an active society (Rare Breeds Conservation Society of New Zealand) registering them, most of the Kune-kune pigs found in New Zealand today is descended from the original 18. Kune-kune pigs have also been exported to the UK, the USA and as far as the European Continent (New Zealand Kune-kune Breeder's Association, available at *http://www.kune-kune.co.nz*).

Results of Australian DNA work presented in 2002 (Gongora *et al.*, 2002), showed that the origins of Kune-kunes could be traced to Asian domestic populations. The Kune-kune (Figure 1.14) developed into its present form in New Zealand, although the pigs are almost certainly of Asian origin (Gongora *et al.*, 2002). During most of the period these pigs have been in New Zealand where they were kept almost solely by Maori communities, and were to a large extent unknown by Europeans. It is quite certain, however, that they were not in this country prior to the arrival of Europeans and they were probably introduced very early in the European period by whalers or traders (Gongora *et al.*, 2002).

Figure 1.14 Representations of typical Kune-kune pigs with their rounded appearance and unique 'tassels'

Individuals of the Kune-kune breed are relatively small and highly distinctive, characterized physically by a short-legged, dumpy build, pot tummy, short upturned nose, and a generally fat and rounded appearance (the Polynesian word *'kunekune'* simply means 'plump'). A unique feature is the 'tassels' which hang from the lower jaw. Kune-kune pigs come in a wide range of colours and are placid animals, easy to maintain, with little tendency to damage pasture.

Exploring the similarities and differences between different indigenous pig populations may lead to a better understanding of genetic variation within and between populations for future viability of livestock.

1.7 FAO Animal Genetic Resource management

There has been much concern in recent years over the loss of biodiversity. Continued genetic improvement of livestock is dependent on the knowledge of the genetic variation that exists within and between populations. The Food and Agriculture Organization (FAO) has set out guidelines for countries to investigate genetic diversity in livestock using genetic markers.

Anthropogenic influences from thousands of years ago to the present day have significantly altered the genetic, distributional and ecological characteristics of many of the world's pig populations (Oliver and Brisbin, 1993). From 1960 – 1990 pig numbers increased by 45% and 201% in developed and developing countries, respectively (FAO, 1992). The increase is largely due to proliferation of a few pig populations and a bigger demand for pork products. While the number of genetic diverse pig breeds may exceed 600 worldwide, there is a limited amount of information to measure their genetic and functional diversity and over 200 populations are considered endangered (Hammond and Leitch, 1998 and Ollivier *et al*., 2001).

The Convention on Biological Diversity (CBD) was established in June 1992 to manage the biological resources on a global scale. Since August 1996, 152 counties have endorsed the CBD and are actively participating in specific objectives agreed upon, namely the conservation of biodiversity, sustainable utilization and the sharing of genetic resources. The Food and Agricultural Organization (FAO) initiated a global action programme, in 1992, to manage the domestic animal genetic resources. Information on the global pig resources in the World Watch List for Domestic Animal Diversity (FAO and UNEP, 2000) illustrate that the continent of Africa has a total of 23 swine populations on the FAO databank DAD-IS (Domestic Animal Diversity Information System, available at *http://www.fao.org/dad-is/htm*) with population data as follows: one breed extinct (Large Black), two populations are critically threatened, two populations are endangered, twelve populations are not at risk and six breed's population data is unknown.

Traditional animal breeding has concentrated on quantitative genetic principles and theory. However, knowledge of the population level variation and genetic population

contribution will promote priority selection for Animal Genetic Resource (AnGR) management. Loss of genetic diversity can be prevented through sensible management by establishing and implementing breeding goals and strategies for sustainable production systems. The *in situ* principle, of the FAO, for indigenous animal genetic resources can benefit future conservation plans (Hammond and Leitch, 1998).

A global management project for the Measurement of Domestic Animal Genetic Diversity (MoDAD) was initiated to help countries design and implement national action strategies, apply microsatellite technology to determine the genetic diversity of pig populations within countries and to compare the data with other countries across the globe, as required under the CBD (Hammond and Leitch, 1998). National focal laboratories are linked to regional focal points that are managed by the Global focus, located at the FAO in Rome. The Animal Genetics laboratory in Irene is regarded as the focal laboratory for the Farm Animal Genetic Resources Programme in the SADC region.

The mission of the global strategy for the Management of Farm Animal Genetic Resources is to (FAO, 2007):

- document existing animal genetic resources,
- develop and improve their use in agriculture,
- maintain those not currently of interest, and
- facilitate access to those animal genetic resources important to food and agriculture.

Animal genetic resources include all species, populations and strains that are of economic, scientific and cultural interest to agriculture, now and in the future. Initially the program will focus on the 14 most important domestic species.

Many local African populations exhibit long-established adaptations to the prevailing climatic, environmental and management conditions and represent a valuable genetic resource for improving the efficiency of animal production. If the breeding work is neglected among such populations or the performance is not recorded and recognised,

populations may be abandoned and lost forever. An understanding of the degree of genetic variation amongst populations can help formulate the construction of breeding programmes and can be used as a tool in the conservation of genetic resources (Mäki-Tanila, 1994).

The critical evaluation of livestock genetic resources and conservation of valuable populations will be important factors in enabling the agricultural and food industries to respond to future changes in consumer needs. Traditional populations may possess potential characteristic genetic variation, which may be valuable to producers in supplying new diversity. Traditional resources may also have the potential to provide for the improvement of commercial pig lines (Blott *et al*., 2003).

Pigs from different populations or breeds vary greatly in size, colour, body shape, ear carriage, behaviour, prolificacy, and other traits. In order to meet future challenges in the agricultural and food industries, special efforts are required to conserve genetic resources. Therefore, phylogenetic studies aimed to evaluate the genetic uniqueness of pig breeds will assist in developing plans for breed conservation programs. Two principles are used in an attempt to select populations important for conservation, namely the degree of endangerment and the genetic uniqueness of the breed (Ruane, 1999).

The status of the genetic diversity of southern African commercial and indigenous pig populations on molecular level is currently unknown (Hammond and Leitch, 1998). Management of genetic resources entails several activities, many of which may greatly benefit from knowledge generated through applying molecular marker technologies for genetic characterization (De Vicente *et al*., 2005).

1.8 Genetic Characterization

Genetic characterization refers to the description of attributes that follow a Mendelian inheritance or that involve specific DNA sequences. In this context, the application of biochemical assays such as those that detect differences between isozymes or protein profiles, the application of molecular markers and the identification of particular sequences through diverse genomic approaches all qualify as genetic characterization methods (De Vicente *et al*., 2005). Molecular characterization also helps determine

the breeding behaviour of species, individual reproductive success and the existence of gene flow, that is, the movement of alleles within and between populations of the same or related species, and its consequences (Papa and Gepts, 2003). Each breed requires a description of its physical characteristics, production traits, information on its distribution, main uses, population numbers, breed-specific indigenous knowledge and the characteristics of the production environment in which it is being used (Laing and Bie, 1998).

It is essential to define, record and measure genetic resources through genetic characterization. This is required at four levels (FAO, 2007):

- base-line survey – a national inventory of animal genetic resources
- monitoring – the population status of farm animal genetic resources
- comparative evaluation – require knowledge of unique qualities of populations and
- comparative molecular description – use of molecular markers to identify significant genetic diversity in populations.

Different molecular techniques have been applied in the past to study the genetic composition of species. In the following section the application of different molecular methods and their contribution to genetic characterization will be discussed.

1.9 Molecular genetic studies on pig populations

Polymorphic genetic markers are measurable characteristics that vary between individuals (Archibald and Haley, 1998). Two types of DNA markers can be identified. Firstly, the DNA-hybridisation markers can be detected through a process of slicing DNA with restriction enzymes, hybridisation with probes and visualising using electrophoresis. Secondly, the PCR-based markers have the advantage of being easier to perform and less time-consuming. Only a small amount of DNA is necessary. A PCR is performed and the segments are transferred directly onto gels, avoiding the need for Southern blotting and hybridisation (Fairbanks and Anderson, 1994b).

During the 1990's combined efforts have been made to construct linkage maps of the porcine genome. In Europe, the PiGMaP (*Pi*g *G*ene *Ma*pping *P*roject) consortium (Archibald, 1994; Haley *et al*., 1994; Archibald *et al*., 1995), the Nordic collaboration (Ellegren *et al*., 1994; Marklund *et al*., 1996) and the USDA Meat Animal Research Centre (Rohrer *et al*., 1994; Rohrer *et al*., 1996) combined efforts and placed more than 1,500 polymorphic genetic markers on the pig linkage map (Archibald and Haley, 1998). The data is freely accessible from *http://www.ri.bbsrc.ac.uk/pigmap/pig genome mapping.html.*

DNA markers (direct or indirect) are essential tools in population genetics, parentage and relatedness analysis, phylogenetics and gene mapping. The different DNA markers and their application in pig genetics are described below under four headings, namely protein studies, fingerprinting methods, sequencing and microsatellites.

1.9.1. Protein studies

1.9.1.1 Blood/Serum Proteins and Enzymes

The first polymorphisms of protein and enzymes were performed during the 1960's (Andresen, 1962; Rasmusen, 1964). These genetic markers are readily available and can be used for different aspects of genetics and breeding, including studies of genetic distance between populations (Klucinski, 1973; Tanaka *et al*., 1983), parentage testing (Gahne and Juneja, 1985), estimation of heterozygosity within populations (Visser and Kotze, 1996), linkage studies (Rohrer *et al*., 1997) and studies on genetic structure of populations and populations (Tao *et al.*, 2005). The level of polymorphism observed in proteins may however be low which has reduced the general application of protein typing in diversity studies (Hanotte and Jianlin, 2005).

1.9.2 Fingerprinting methods

1.9.2.1 Variable Number of Tandem Repeats (VNTR markers) or DNA Fingerprints

Variable Number of Tandem Repeats is short sequences (10-100 base pairs) of chromosomal DNA that are repeated many times. The number of times that a sequence is repeated varies between different individuals and between maternal and paternal loci of an individual. The location and length of VNTR sequences is unique

to each individual. DNA flanking a VNTR is cut with a restriction endonuclease. The size of the resulting DNA fragment can vary and is visualized through DNA hybridization. Multi-locus VNTR probes yield a genetic fingerprint pattern which is a useful forensic tool (Fairbanks and Anderson, 1994a).

Minisatellites have fallen into disfavour because they are not suitable to PCR due to the large repeat size. These markers tend to be more prominent towards the telomeric regions of chromosomes (Hetzel and Drinkwater, 1992). Minisatellites are nevertheless useful in genetic relationships (Coppieters *et al*., 1995), linkage studies (Signer *et al*., 1996) and population structure (Signer and Jeffreys, 1997; Signer *et al*., 2000).

1.9.2.2 Restriction Fragment-Length Polymorphism (RFLP Markers)

Restriction fragment-length polymorphism analysis relies on differences in DNA sequences that affect the position of restriction enzyme recognition sites in the DNA. Sample DNA is digested with one or more restriction enzymes and the resulting fragments are separated according to molecular size using gel electrophoresis. Molecular size standards are used to estimate fragment size. The "target" sequence is hybridized with the correct probe and then visualised using the Southern blot technique. This results in a multi-fragment DNA pattern. The advantage of RFLPs is that it can be used to screen a large number of individuals without involving complicated molecular techniques (Anderson and Fairbanks, 1994a)

Gene mapping (Sun *et al*., 1998) and linkage studies (Zuo *et al*., 2005) in pigs were performed using RFLP technology. The greatest drawbacks of RFLPs are their diallelic properties and the information content is generally low for within species analysis (Archibald and Haley, 1998).

1.9.2.3 Amplified Fragment-Length Polymorphism (AFLP Markers)

Amplified Fragment-Length Polymorphism is a PCR-based fingerprinting technique which, in contrast to most other random amplification techniques, makes use of stringent PCR conditions. It involves the restriction of genomic DNA, followed by ligation of adaptors complimentary to the restriction sites and selective PCR amplification of a subset of the adapted restriction fragments. These fragments are

visualized on denaturing polyacrylamide gels either through autoradiographic or fluorescence methodologies (Vos *et al*., 1995). AFLPs provide an effective, rapid and economical tool for detecting a large number of polymorphic genetic markers that are highly reliable and reproducible, and can be genotyped automatically (Jun *et al*., 2004).

A potential limitation of AFLP is the degree to which both alleles can be detected at any given locus. These markers are less informative for linkage analysis than co-dominant markers (Ovilo *et al*., 2000a). The AFLP technique has been used extensively to detect genetic polymorphisms, evaluate and characterize breed resources, construct genetic maps and identify genes. Studies of population genetic relationships among different pig populations were measured through AFLP markers technology by Ovilo *et al.* 2000b), Ciobanu *et al.* (2001) and Ren *et al.* (2002). Another study applied AFLP markers to detect quantitative trait loci in a pig carcass (Wimmers *et al*., 2002) as a method for detection of genome regions containing QTL in livestock. Other studies revealed that AFLP markers could be applied to determine genetic relationships between and within pig populations (Kim *et al*., 2002) and to discriminate between different lines (Cameron *et al*., 2003).

1.9.2.4 Random Amplified Polymorphic DNA (RAPD Markers)

RAPDs are DNA fragments amplified by PCR using short arbitrary primers (10-15 base primers). After running these segments on an agarose gel, a banding pattern of different sized segments are produced, some of which include samples of interest as well as many other samples that need to be excluded.

The patterns of bands may be different for individuals in a population. These markers can be used for linkage studies and *in situ* hybridisation. It is fast and very cost efficient. It does not require prior sequence knowledge, requires nanogram amounts of template DNA and a minimum of laboratory equipment (Anderson and Fairbanks, 1990). It is highly suitable for genetic diversity and phylogenetic relationship studies (Chang *et al*., 1999), quick fingerprinting (Stift *et al*., 2003) and the identification of species origin (Arslan *et al*., 2005).

In an example of application of this technique in pigs, the RAPD technique was used for the identification of diagnostic markers that allowed the detection of Duroc alleles in Iberian pig samples (Ovilo *et al*., 2000a). RAPD markers, however, are inherited as dominant and recessive alleles and could present repeatability problems.

1.9.3 Sequencing Studies

1.9.3.1 Sequencing of mitochondrial DNA (mtDNA)

Mitochondria are cellular organelles within the cytoplasm of cells which reproduce autonomously. Mitochondria has been widely used for phylogenetic studies for several reasons: evolution of mammalian mtDNA occurs primarily as single base pair substitutions; the rate of evolution appears to be up to 10 times faster than nuclear DNA and mtDNA is maternally inherited. The displacement (D) loop region of mtDNA is known to be more variable in sequence than other regions and frequently used for phylogenetic studies (Moran, 1998; Kim *et al*., 2002).

The complete mtDNA sequence of the pig was published by Ursing and Årnason in 1998. Data from mtDNA was also used to determine the phylogenetic relationships among Chinese pig populations (Lan and Shi, 1993) and between Iberian and Spanish pig populations (Alves *et al*., 2003). The origin of domestication in Eurasian and European pig populations (Giuffra *et al*., 2000; Larson *et al*., 2005) was determined by using mtDNA technology. Another study assessed the phylogenetic relationship of the Kune-kune and Auckland Island pig populations in New Zealand by comparing their mitochondrial D-loop DNA sequences to determine the origins of each breed (Gongora *et al*., 2002).

1.9.4 Single Nucleotide Polymorphisms (SNPs)

Single nucleotide polymorphism technology is a recently developed technique. Microarray technology allows the simultaneous analysis of thousands of parameters within a single experiment, thus generating large amounts of genomic data (Templin *et al*., 2002). A SNP is a single base substitution of one nucleotide with another. It is estimated that every 100-300 nucleotide in the genome is polymorphic. SNPs can occur in both coding and non-coding regions of the genome. By studying SNP profiles, researchers may begin to identify relevant genes associated with disease.

SNPs are responsible for much of the genetic variation within a species. They are also evolutionarily stable - not changing much from generation to generation - making these markers easier to follow in population studies (Eding and Laval, 1999). For a SNP to be considered a variation, it must occur in at least one per cent of the population.

SNP technology is useful in gene expression in different populations (Jiang *et al*., 2001; Fahrenkrug *et al*., 2002; Munoz *et al*., 2004). SNPs also offer value in forensic research (Gotaro *et al*., 2004), genetic traceability (Goffaux *et al*., 2005), identification and parentage exclusions (Rohrer *et al*., 2007) and population genetics (Chen *et al*., 2007).

1.9.5 Microsatellite markers

The abundance and ubiquitous distribution of microsatellites make these genetic markers very valuable. Microsatellites have become the marker of choice for linkage mapping, population diversity measurements, calculation of genetic distances, genetic relationships, individual identification and inbreeding estimation (Takazaki and Nei, 1996; Xu and Fu, 2004). A detailed description of the applicability of microsatellite markers during the current study is presented in the following section.

1.9.5.1 Application of microsatellites

Microsatellites are short tandem repeat polymorphisms (STRs). The repeated unit can be a mono-, di-, tri- or tetranucleotide with di-repeats being most common. They generally occur in non-coding regions of the genome. On each side of the repeat unit are flanking regions that are critical because these regions allow for the development of unique primers. The mutation process in microsatellites occurs through what is known as intra-allelic polymerase slippage replication (Bruford *et al*., 1996).

The repeat units are short (one to six nucleotides) and have reasonably small copy numbers, which makes them suitable for PCR amplification. Microsatellites are very abundant (estimated at 65,000–100,000 loci in the porcine genome) and spread over the entire genome of all living organisms, so markers can be readily developed for

any genetic objective (Moran, 1993). Microsatellite primers developed for one species frequently amplify loci in related species (Wright and Bentzen, 1994). Microsatellites have high mutation rates (between 7.52 x 10^{-4} and 4.08 x10^{-5} per locus per generation) and therefore may show high variation between individuals within a species (Yue *et al*., 2002).

1.9.5.2 Advantage of microsatellites as genetic markers

In contrast to multi-locus markers such as minisatellites or RAPDs, microsatellites are co-dominant markers and individuals can be classified as heterozygotes or homozygotes at a given locus. Since microsatellites are PCR-based only tiny amounts of biological material are needed to provide input DNA and highly degraded or "ancient" DNA can produce successful results. These markers are highly polymorphic ("hypervariable") and provide considerable PIC (polymorphic information content). For individual identification to fine-scale phylogenies, these markers are useful at a range of scales. Microsatellites can also be applied across a wide range of related taxa (Estoup *et al*., 1993; Engels *et al*., 1996; Cornuet *et al*., 1999; Primmer *et al*., 2003; Peacock *et al*., 2004; Hoda *et al*., 2009).

1.9.5.3 Limitations of microsatellites

Limitations include reports that for certain groups of organisms microsatellites are difficult to isolate (Beaumont and Bruford, 1999), the technical challenges of microsatellite analysis for some types of samples such as saliva, hair or faecal material (Gerloff *et al*., 1995; Taberlet *et al*., 1996; Gagneux *et al*., 1997) and the fact that data generated in different laboratories using different methods have at times proved difficult to combine (Beaumont and Bruford, 1999).

The development of microsatellite markers is a tedious process and comparatively few laboratories in South Africa have the necessary expertise to produce entirely new markers. The over-all cost for microsatellite analysis is also relatively high, considering the cost of the fluorescent labelling of primers and the optimisation necessary to adjust the PCR technique. New technology based on pyro-technology may however address some of the limitations discussed above.

Furthermore the occurrence of “null” alleles has been discovered in some offspring as non-inheritance of parental alleles. A deletion or insertion may also occur in the flanking primer regions. Heterozygous individual can then be mistyped as homozygotes. Another limitation is that *Taq* DNA polymerase may attach an Adenosine nucleotide (A) to the 3’ end of amplified fragments that can create inaccurate allele identification (Ciofi *et al*., 1998). Nevertheless, several studies on pig populations have been done in the past decade using microsatellite markers for different molecular results. The different studies will be discussed in detail in the following section.

1.9.5.4 Examples of microsatellite studies on pig populations

Microsatellites were used in different studies on pig populations, mostly in Asia and Europe with the emphasis on genetic diversity of pig populations. An extensive discussion regarding these studies follows as it is important to compare the data of the current study to that of previous studies on pig populations from different countries. A summary of the results from the genetic measures observed in different studies, *i.e.*, the number of microsatellite markers used, average number of observed alleles, F_{ST}-, F_{IS}- and expected heterozygosity (He) values, where available, as well as the resources is indicated in Table 4.1 (Chapter 4).

In Europe, different studies have been conducted using blood samples from various pig populations. A presentation of the different studies on European pig populations and the results obtained from these studies follow.

A genetic study was performed on four Belgian pig populations (n=750) using seven microsatellite loci (Van Zeveren *et al*., 1995). The mean number of observed alleles was 9.075 and the effective number of alleles was 2.95. The F_{ST} value was 0.32 (0.181-0.425). The average expected heterozygosity was 0.59. This study rather focused on exclusion probabilities and efficiency in parentage control. According to this study, the Belgian Landrace and Belgian Negative pigs were the closest related, while the Piétrain was the most distant breed.

Genetic diversity of eleven European pig populations (n=483) from six European countries was performed using 18 microsatellite markers (Table 1.7) recommended by

the FAO-ISAG advisory Committee (Laval *et al*., 2000). The mean observed allele number was 4.6 and the effective number of alleles was 2.21. The F_{IS} value was 0.052 (0.007-0.239) and the F_{ST} value was 0.27 (0.12-0.74). Two European populations illustrated significant deviations from Hardy-Weinberg proportions due to the high positive F_{IS}. The panel of markers used in this study exhibited high polymorphisms and the eleven European populations indicated strong differentiation. The authors found it difficult to describe a reliable phylogeny among the populations, since the present domestic breeds have not resulted from a strict tree-like branching process. The importance of global evaluations of diversity of populations worthy of preservation was also pointed out by Laval *et al*. (2000).

Fabuel *et al.* (2004) performed a genetic diversity study on five Iberian pig populations (n=173) based on 36 microsatellites recommended by the FAO-ISAG Advisory Committee. Duroc pigs (n=40) were also included in the study due to the historical relationship of this breed with the Iberian pigs. The average number of alleles for the Iberian pigs was 7.2 and 5.4 for the Duroc population. The average He for the Iberian pigs was 0.697 and 0.648 for the Duroc pigs. The F_{ST} value among breeds was 0.129 and the average F_{IS} value was 0.045. This study demonstrated a high level of genetic diversity in the Iberian pig populations. Furthermore, the Iberian and the Duroc populations show a clear differentiation based on genetic distances.

A study of the Turopolje pig breed (n=250) of Croatia demonstrated low genetic diversity (Harcet *et al*., 2006). There is little known about the genetic background of this pig breed. The findings are attributed to a severe demographic bottleneck experienced in the middle of the 20th century and the possibility that the Turopolje pig was domesticated locally. Ten microsatellite markers were selected according to the FAO-ISAG Advisory Committee (Table 1.7). The mean observed allele number was 2.4 and the effective number of alleles was 1.47. There were no significant deviations from Hardy-Weinberg equilibrium. The average He was 0.272.

In a study by SanCristobal (2006), genetic diversity was determined within and between 58 European pig populations and a Chinese (Meishan) breed (n=2737) using 50 microsatellite markers. The mean observed allele number was 4.50 and the effective number of alleles was 2.74. The average F_{IS} value was 0.013 (0.005-0.023) and the average F_{ST} value was 0.21 (0.20-0.22). Fifteen European populations

presented significant deviations from Hardy-Weinberg equilibrium with an excess of homozygotes due to the “wahlund” effect. The average He was 0.56. Significant clustering of lines within main populations (Large White, Landrace, Duroc, Hampshire and Piétrain) was found while inbreeding has had a substantial impact on between-breed diversity of European breeds.

Rodrigáñez *et al*., (2008) performed a study on Spanish wild (n=68) and domestic (Iberian and Duroc) pig breeds (n=234) using eighteen microsatellite markers. The mean observed allele number was 5.3. The F_{ST} value was 0.687 (0.408-0.712). The average expected heterozygosity was 0.61. The wild boar and Duroc populations presented the highest number of private alleles, compared to the Iberian pigs, and ranked together according to their contribution to diversity.

Extensive genetic studies have also been performed on pig populations in Asia. The findings of these studies are described in the following paragraphs.

The genetic structure of seven Chinese indigenous pig populations (n=380) were investigated using the 27 microsatellites (Table 1.7) recommended by FAO-ISAG (Fan *et al*., 2002). The average number of alleles was 4.92 and the effective number of alleles was 2.82. The average expected heterozygosity was 0.591. Significant departure from Hardy-Weinberg equilibrium was observed, probably caused by founder effects, intensive selection and close breeding. The average F_{ST} value was 0.18 (with a range of 0.016-0.318). Previously published data on European pigs were also included and this confirmed that Chinese indigenous pigs and European pigs have diverged into two distinct groups, based on genetic distance analysis.

A study by Fan (2003) of four Chinese miniature pig populations, revealed a genetic distinctive cluster when compared to the Duroc pig breed. The average number of alleles for the Chinese pigs was 5.30 with the effective number of alleles 4.12 and the average number of alleles for the Duroc pigs was 3.67 with the effective number of alleles 2.59. The average expected heterozygosity for the Chinese pigs was 0.672 and 0.493 for the Duroc pigs.

Genetic variation of eighteen Chinese indigenous pig populations (n=1001) was performed using 26 microsatellites (Table 1.7) recommended by the FAO-ISAG Advisory Committee by Yang *et al*. (2003). Three commercial (Large White,

Landrace and Duroc) pig populations (n=184) were used as a reference group. The average number of alleles was 13.31 and the effective number of alleles was 7.44. The average expected heterozygosity was 0.842. The loci deviated from HWE ranged from 1 to 6 in each population and were the effect of the sample collection. The genetic basis of the pigs was narrow and the founder effect could have an influence on the population. The mean F_{ST} value was 0.077 (0.019-0.170) and the average F_{IS} value was 0.274 (0.001-0.481). It was evident through this study that there is an abundance of genetic variation in Chinese indigenous pig populations but that the genetic distance between these populations are quite low. The European populations clustered on a different branch.

The genetic diversity of ten indigenous Chinese pig populations and one exotic Duroc pig breed (n=403) was determined by Li *et al*. (2004). The average number of alleles for the Chinese pigs was 8.61 with the effective number of alleles 4.39 and the average number of alleles for the Duroc pigs was 7.5 with the effective number of alleles 2.21. The average expected heterozygosity for the Chinese pigs was 0.68 and 0.47 for the Duroc pigs. The mean F_{ST} value was 0.22 (0.05-0.37) and the average F_{IS} value was 0.21 (0.08-0.48). Deviations from Hardy-Weinberg equilibrium were observed, with excesses of homozygotes du to the "Wahlund" effect. This study demonstrated that large genetic differentiation exists in the particular Chinese populations studied.

Ankamali pigs of India were genetically characterized using 23 FAO-ISAG recommended microsatellites (Table 1.7) and were compared with three other native Indian pig populations (n=26) and a Large White pig breed (n=45) by Behl *et al*. (2006). The mean observed allele number was 7.0 and the effective number of alleles was 5.11. The mean F_{ST} value was 0.06. The average expected heterozygosity across populations was 0.83. The Ankamali breed was found to be very distinct compared to the other pig populations.

A genetic variation study of Lanyu and six exotic pig populations (n=1250) in Taiwan was performed using nineteen FAO-ISAG recommended microsatellite markers by Chang *et al*. (2009). The mean observed allele number was 3.74 and the effective number of alleles was 2.39. The mean F_{IS} value was 0.332 (0.020-0.858) and the average F_{ST} value was 0.398 (0.217-0.605). The average expected heterozygosity was

0.559. Deviations from Hardy-Weinberg Equilibrium were observed with a significant loss of heterozygosity due to genotyping error, null alleles and population substructure. The Lanyu pig possessed a unique genetic signature and was thus shown to be very distinct from both European and Asian domestic populations.

An analysis of diversity and genetic relationships between four Chinese indigenous pig populations (n=61) and one Australian commercial pig breed (n=30) was performed using 27 FAO-ISAG (Table 1.7) recommended microsatellites by Li *et al*. (2000). The mean observed allele number was 3.87. The average expected heterozygosity was 0.703. The Australian pig population was genetically distant from the four Chinese populations. A full report was presented on the use of microsatellites recommended by the FAO-ISAG Committee as tool for the classification and conservation of indigenous populations and it is recommended that the use of the recommended microsatellite panel should be applied by other laboratories for comparability studies.

A few comparative studies between European and Asian pig populations have also been carried out. Kim *et al*. (2005) reported on a study between two Korean and three Chinese pig populations (n=116) using 16 microsatellite markers. Four European pig populations (Berkshire, Duroc, Landrace and Yorkshire) (n=126) were included in the study. The mean observed allele number was 4.67. The mean F_{ST} value between populations was 0.261 (0.196-0.331) and the average F_{IS} value was 0.067 (0.012-0139). The average expected heterozygosity was 0.613. Deviations from HWE were observed based on heterozygote deficiencies and could be due to the “Wahlund” effect. The Korean native pig had low genetic diversity and clustered close to the European pigs since it was crossed with commercial breeds to improve their productivity. The South China populations were genetically distant from all other populations.

A comparative study of seven Vietnamese and three European pig populations (n=343) using 20 microsatellite markers, were reported by Thuy *et al*. (2006). The mean observed allele number was 6.45 and the effective number of alleles was 5.14. The mean F_{ST} value was 0.050 (0.019-0.138). The average expected heterozygosity in populations was 0.559. The Vietnamese indigenous populations demonstrated an abundant genetic diversity reservoir compared to the European populations.

Ten microsatellites (FAO-ISAG recommended – Table 1.7) were used to determine the genetic distinctiveness of seven Mexican hairless pig populations (n=177) by Lemus-Flores *et al*. (2001). Four commercial pig populations (n=111) were also included in this study. The average number of alleles was 13.8 with a high of 6.8 in the Mexican hairless populations. The average expected heterozygosity in populations was 0.73. Most populations deviated from Hardy-Weinberg proportions with an excess of homozygotes due to the "Wahlund" effect. The mean F_{ST} value was 0.10 (0.08-0.16) and the average F_{IS} value in populations was 0.25 (0.04-0.61). This study revealed that the Mexican hairless pig is a unique population highly distant from commercial pig populations.

Microsatellites are also useful in determining origin, as is shown in the study by Fan (2005) where population genetic variability and origin of the Auckland Island feral pigs were determined. A panel of 26 microsatellite markers, recommended by the FAO-ISAG Advisory Committee (Table 1.7) was used. Data from 21 Auckland Island feral pigs were compared to previously published data of European, Asian and Australian pig populations. The mean observed allele number was 3.654 and the affective number of alleles was 2.175. The average expected heterozygosity was 0.454. The average F_{IS} value was 0.168. It was determined that the genetic variation within the Auckland pigs was lower than that these pigs may be confronting critical challenges from genetic diversity loss. The Auckland pigs consistently clustered with the European lineages. This study successfully confirmed the use of microsatellite markers in understanding the origins and structure of the Auckland pig populations as it verified findings using mitochondrial data.

A study to assess the genetic diversity and structure within and among wild pigs from Australia and Papua New Guinea (n=320) was performed by Spencer *et al*. (2006). This study also included a number of commercial pig populations (Large White and Landrace). Fourteen microsatellite loci were used. The mean observed allele number was 5.82. The average expected heterozygosity was 0.758. The mean F_{ST} value among populations was 0.159. High levels of genetic variation was found, suggesting that feral pig populations from Australia and Papua New Guinea contain substantial genetic information not contained in the commercial pig populations included in this study. Populations also showed a substantial degree of differentiation from one another.

Table 1.7 A panel of 27 microsatellite markers, recommended by the FAO-ISAG Advisory Committee (Source: Laval *et al*., 2002 and Fan *et al*., 2005)

Marker	Chromosomal position	Allele length
CGA	1p	280-302
S0155	1q	150-164
SW240	2p	97-111
S0226	2q	181-205
SW72	3p	98-112
S0002	3q	199-215
S0227	4p	230-234
S0005	5q	222-244
IGF1	5q	202-206
SW122	6q	116-126
S0228	6q	227
SW632	7q	159-170
S0101	7q	206-216
S0225	8q	182-188
S0178	8q	110-124
SW911	9p	158-168
SW951	10q	124-126
S0386	11q	150-164
S0090	12q	242-251
S0068	13q	240-256
S0215	13q	157-171
SW857	14q	150-160
S0355	15q	247
SW936	15q	98-118
S0026	16q	98-104
SW24	17q	102-110
S0218	Xq	168-190

For the purpose of the current study, microsatellite markers was therefore chosen as the marker of choice based on the advantages it presents compared to other markers. These are: (i) high rates of polymorphisms; (ii) ease of use; (iii) repeatability; (iv) accuracy (v) the degree of polymorphic information content and (vi) availability (Bruford and Wayne, 1993). A panel of 52 microsatellite markers were donated courtesy of Professor Max Rothschild from the Iowa State University (Pig Genome Coordination Project of the US Department of Agriculture).

1.10 Broad objective of the research programme

Very limited information on the genetic variability measurement and genetic differences of the southern African pig populations exists. The purpose of this study was to participate in the genetic characterization of farm animal genetic resources in the southern African Development Community (SADC) region. It would also provide information as to which of the populations represent homogeneous breeds and are therefore genetically distinct. Further information will contribute to the determination

of the risk status and conservation and management of pig genetic resources. Ultimately, the knowledge gained from this study will contribute to the understanding of the development history of pigs in southern Africa.

1.11 Aims of the Study

This is the first study initiated to document the genetic characterization of local pig populations using microsatellite markers and to provide an overall representation of their genetic diversity in South Africa. The specific aims of this project include:

1. Optimization and validation of a set of microsatellite markers, for the genetic characterization of southern African commercial and indigenous pig populations.

2. Genetic characterization of the three principal commercial pig populations (the Landrace, the Duroc and the Large White) of South Africa and the indigenous pig populations (the Kolbroek, Mozambican and Namibian pig populations) of southern Africa using selected microsatellite markers.

Contributing to the global genetic characterization of indigenous farm animals and assessment of the genetic variation of other pig populations in South Africa will lead to the optimization of a microsatellite marker set that can be applied to (i) individual identification (DNA profiling); (ii) breed characterization; (iii) parentage verification and (v) forensic science.

CHAPTER 2

Materials and Methods

2.1 Populations

In the study presented here, a total of seven pig populations were selected for genetic characterization. In South Africa, the SA Landrace, Large White and the Duroc were selected to represent the commercial pig populations. These pigs have specific phenotypic traits (see Section 1.3) that are comparable to international breed standards. The Namibian, Mozambican and Kolbroek pigs were selected to represent the indigenous pig populations of specific regions. The Namibian pigs phenotypically resemble the "Kolbroek"-type pig and the Mozambican pigs phenotypically resembles the rural 'Hut" pig. These populations were selected to be representative of the total pig population in southern Africa for assessment of their genetic status. Another pig population, the Kune-kune from New Zealand, were selected as the reference group. The Kune-kune pigs phenotypically resembles the "kolbroek"-type pig. The term "population" is used as an alternative to "breed" in the case of the indigenous pig breeds, since the uniqueness of these groups has not yet been determined.

2.2 Samples and localities

The aim was to sample 10 unrelated males and 30 unrelated females. This however was not always possible for all the populations sampled. The samples were presumed to be unrelated, after verification of the data obtained from the different breeder societies and facility managers involved. Ideally, biological samples should be collected using a scientifically structured method of randomly selected unrelated individuals. In order to obtain the most unbiased sample for each population during the current study, an approach was followed in which samples were received from a variety of sources and from different geographical regions with information on the origin, phenotypical traits and pedigree of the individuals.

Hair samples of pigs were selected as source material for DNA extractions for the advantages it has in terms of collection and assay purposes in contrast to blood and tissue collection. These samples are easy to collect. Care should however be taken that the hair follicles are visible, clean and dry. Hair samples can be stored for long periods if it is kept dry and away from sunlight. Hair samples were thus simply plucked from the backs of pigs and stored in an envelope or plastic bag. Each bag contained an information card with the following details provided by the sender: animal identification, gender, pedigree (if known), breed, origin, owner's contact

information and collection date.

A total of 350 hair samples with visible roots were collected from the seven pig populations used for this study. Samples were collected from stud animals at Artificial Insemination (AI) stations, farmers submitting samples for Malignant Hypothermia (MH) testing and from other studies, *i.e.*, swine fever testing. A total of 21 hair samples from the Kune-kune breed of New Zealand were also obtained for DNA analysis. These individuals were included to compare differences between southern African indigenous populations and established commercial populations with differences between the latter and unrelated established populations. Permission to use the genotypic data of the Kune-kune populations was obtained from the New Zealand Government as the data was only used as reference material. The total number of samples collected from the different populations and different localities is summarized in Table 2.1 and the distribution of sample collection is presented in Figure 2.1.

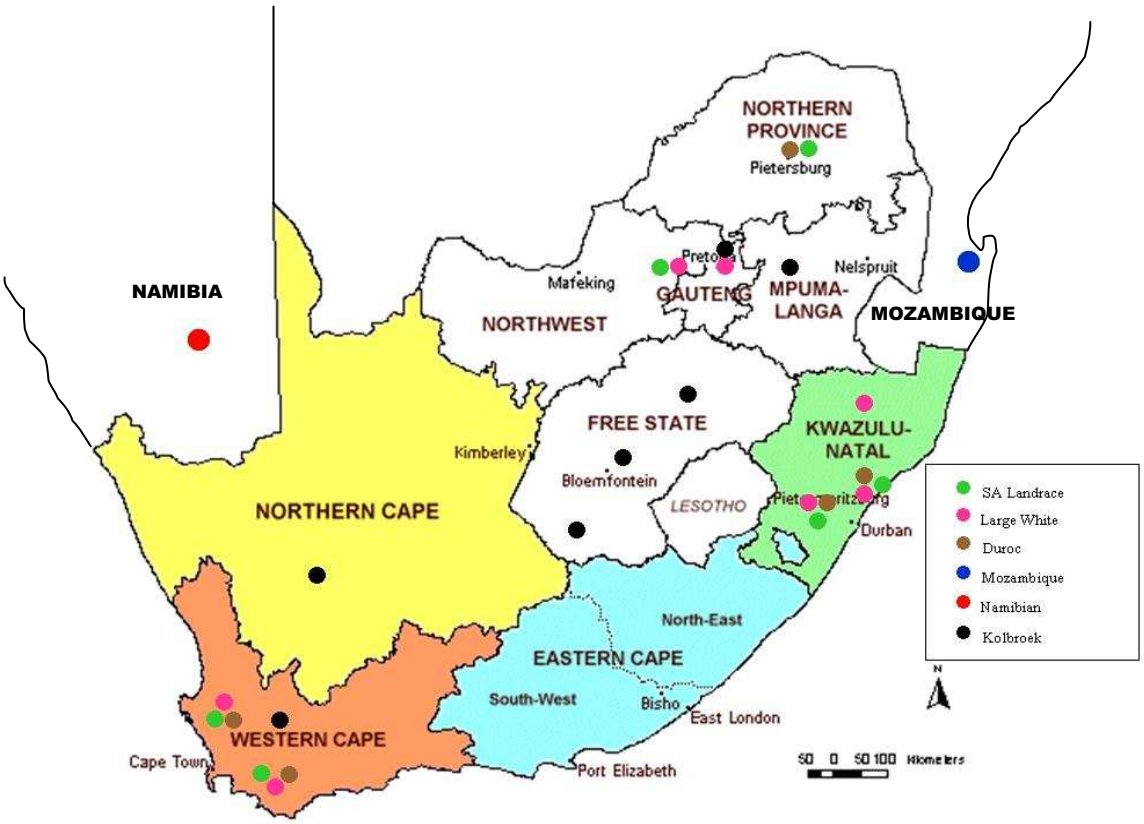

Figure 2.1 The origin of pig samples collected throughout southern Africa

Table 2.1 The total number of pigs sampled from different localities

Breed	Locality	Number of Samples
SA Landrace	Stellenbosch, Western Cape	5
	Howick, KwaZulu Natal	8
	Mooi River, KwaZulu Natal	3
	Polokwane, Limpopo	3
	Magaliesburg, North West	3
	Somerset-West, Western Cape	3
Large White	Stellenbosch, Western Cape	5
	Baynesfield, KwaZulu Natal	8
	Pretoria, Gauteng	3
	Howick, KwaZulu Natal	8
	Magaliesburg, North West	4
	Somerset-West, Western Cape	3
Duroc	Stellenbosch, Western Cape	4
	Howick, KwaZulu Natal	8
	Mooi River, KwaZulu Natal	3
	Polokwane, Limpopo	3
	Magaliesburg, North West	1
	Somerset-West, Western Cape	3
Indigenous	Namibia – Rundu (Kavango region)	24
	Mozambique - Angonia (Tete Province)	152
Kolbroek	Irene, Gauteng	33
	Eastern Cape	9
	Western Cape	4
	Sasolburg	1
	Chrissiesmeer	7
	Rouxville	6
	Hoekville	7
	Mosselbay	8
Kune-kune	New Zealand	21
TOTAL		**350**

2.3 Methods

The method used for Deoxyribonucleic Acid (DNA) extraction is cost-effective and easy to perform, as described below. PCR optimization, electrophoresis, data collection and statistical analysis are also described in detail in the following section.

2.3.1 DNA extraction

The method of choice was to perform the extraction of genomic DNA from hair roots. It was based on a modified method by Higuchi (1988). The hair roots (2 mm portions) were clipped with sterilized scissors into an autoclaved 1.5 ml microcentrifuge tube. When the results were analyzed on an agarose gel, the intensity of the bands

suggested that 5 to 9 hairs were needed to provide sufficient template DNA for a PCR reaction depending on the condition of the hair. The DNA extraction method was easily applied and cost effective, and yielded approximately 20 ng/µl DNA per sample using a spectrophotometer. DNA extraction solution for a n+1 reaction was prepared as follows: distilled water, 4 mg/ml Proteinase K, 2 mM Supertherm Gold Buffer™ (20 mM Tris-HCl pH 8.3, 15 mM $MgCl_2$, 50 mM KCl) and Tween100 (undiluted). A volume of 100 µl of the solution was pipetted into each tube, ensuring that the hair roots were completely immersed in the solution during heat treatment, and was then incubated at 56°C for two hours. Following incubation, tubes were vortexed for 5-10 seconds and kept at 100°C for 10 minutes to deactivate the Proteinase K. The samples were then centrifuged for 2-3 minutes at 13,000 rpm and the supernatant stored at -20ºC or used directly as template in the PCR reaction.

2.4 Microsatellites

There are many different molecular techniques to assess the genetic status of populations, e.g., blood/serum proteins and enzymes, RFLPs, RAPDs, AFLPs, mtDNA, microsatellites, SNP and QTL technologies. In this study, the method of choice was microsatellites markers based on the advantages of having high rates of polymorphisms, the ease of use, their repeatability and accuracy, as well as the degree of polymorphic information content and their availability (Bruford and Wayne, 1993).

2.4.1 PCR amplification

A panel of 52 microsatellite loci distributed throughout the genome and identified as part of the Pig Genome Project was initially used in this study (Table 2.2). The primers were obtained in lyophilized form and were already fluorescently labeled on the 5' end of the forward (sense strand) primer. 40 of the markers were eventually selected based on successful amplification and their proven properties as DNA markers (see Section 1.8). The primer name and sequence for each of the microsatellite loci are summarized in Table 2.3.

Table 2.2 A Table to illustrate the microsatellite markers used, their position on chromosomes, the fluorescent dyes used and relevant references

Locus	*Chromosome position*	*Fluorescent dye*	*References*
S0073	4	Fam	-Rohrer *et al*., 1996 -Fredholm *et al*., 1993
SW35	4	Fam	-Rohrer *et al*., 1996 -Rohrer *et al*., 1994
S0298	16	Tet	-Rohrer *et al*., 1996 -Hoyheim *et al*., 1994
SW1134	5	Tet	-Rohrer *et al*., 1996 -Rohrer *et al*., 1994
SW1851	1	Tet	-Rohrer *et al*., 1996 -Alexander *et al*., 1996 -Lopez-Corrales *et al*., 1999
SW2456	Y/X	Hex	-Rohrer *et al*., 1996 -Alexander *et al*., 1996
SW2514	2	Hex	-Rohrer *et al*., 1996 -Alexander *et al*., 1996
SW983	9	Hex	-Rohrer *et al*., 1996 -Rohrer *et al*., 1994
S0120	18	Fam	-Rohrer *et al*., 1996 -Groenen *et al*., 1995
SW2	5	Fam	-Rohrer *et al*., 1996 -Rohrer *et al*., 1994
SW1557	14	Tet	-Rohrer *et al*., 1996 -Alexander *et al*., 1996
SW378	5	Tet	-Rohrer *et al*., 1996 -Rohrer *et al*., 1994 -Lopez-Corrales *et al*., 1999
SW761	14	Tet	-Rohrer *et al*., 1996 -Rohrer *et al*., 1994
S0038	10	Hex	-Rohrer *et al*., 1996 -McQueen *et al*., 1994
SW2008	11	Hex	-Rohrer *et al*., 1996 -Alexander *et al*., 1996
SW995	5	Fam	-Rohrer *et al*., 1996 -Rohrer *et al*., 1994
SW352	7	Tet	-Rohrer *et al*., 1996 -Rohrer *et al*., 1994
SW472	7	Tet	-Rohrer *et al*., 1996 -Rohrer *et al*., 1994
SW949	Y/X	Tet	-Rohrer *et al*., 1996 -Rohrer *et al*., 1994 -Lopez-Corrales *et al*., 1999
S0004	15	Hex	-Rohrer *et al*., 1996 -Fredholm *et al*., 1993
S0165	3	Fam	-Rohrer *et al*., 1996 -Ellegren *et al*., 1994
S0217	4	Tet	-Rohrer *et al*., 1996 -Robic *et al*., 1994
SW225	13	Tet	-Rohrer *et al*., 1996 -Rohrer *et al*., 1994
SW1041	10	Hex	-Rohrer *et al*., 1996 -Rohrer *et al*., 1994 -Lopez-Corrales *et al*., 1999

Table 2.2 (Continue)

SW21	9	Hex	-Rohrer *et al*., 1996 -Alexander *et al*., 1996 -Lopez-Corrales *et al*., 1999
SW2404	4	Hex	-Rohrer *et al*., 1996 -Alexander *et al*., 1999
S0035	6	Fam	-Rohrer *et al*., 1996 -Brown and Archibald. 1995
S0006	16	Tet	-Rohrer *et al*., 1996 -Fredholm *et al*., 1993
SW749	9	Tet	-Rohrer *et al*., 1996 -Rohrer *et al*., 1994
SW2410	8	Hex	-Rohrer *et al*., 1996 -Rohrer *et al*., 1994 -Lopez-Corrales *et al*., 1999
SW940	9	Hex	-Rohrer *et al*., 1996 -Rohrer *et al*., 1994
S0295	9	Fam	-Rohrer *et al*., 1996 -Hoyheim *et al*., 1994
SW839	4	Fam	-Rohrer *et al*., 1996 -Rohrer *et al*., 1994
SW2406	6	Tet	-Rohrer *et al*., 1996 -Alexander *et al*., 1999
SW2419	6	Tet	-Rohrer *et al*., 1996 -Alexander *et al*., 1999
SW316	6	Hex	-Rohrer *et al*., 1996 -Rohrer *et al*., 1994
S0121	7	Fam	-Rohrer *et al*., 199 -Robic *et al*., 1994
SW322	6	Tet	-Rohrer *et al*., 1996 -Rohrer *et al*., 1994
S0385	11	Hex	-Rohrer *et al*., 1996 -Riquet *et al*., 1995
SW2443	2	Hex	-Rohrer *et al*., 1996 -Alexander *et al*., 1999

Table 2.3 Sequences of 40 microsatellite markers used for the genetic characterization of pig populations (5' – 3' in all instances)

Locus	***Reverse primer***	***Forward primer***
S0073	ACTGAAACAGGAATTCAGATCC	TGAAGTATTATGGCATCATGGA
SW35	TCAAGTTGGAGAGTCTGAGGC	AAGACTGCCCACCAAATGAG
S0298	ACATAACATCGTAAATCAGC	CTCCATCACAGGTCTCACA
SW1134	TAAGTTTAGGTGCCTCATTTGATTT	GAAAACTCTCTTAGTTTCTTTATGCA
SW1851	GGCTTGGACATTCTCATTGG	GGTTGAGGAACCCTGATGTG
SW2456	GAGCAACCTTGAGCTGGAAC	AATGTGATTGATGCTGTGAAGC
SW2514	CATGTGCTGGTCAGGCAG	AAGGAGGTGACCGTGTGG
SW983	GCAGTCCCACTCTTAGGTATATATCC	ATAATGCTGCTATGAACACTGTAGTG
S0120	GCCTAAGTAGAATTAAGCACAAGG	GTGCTCTCACTGCCTTCATATACC
SW2	TGCCAATGGTGTGGCTATAA	CCCTGAAGGCTCAGATGGT
SW1557	TGCTCTAATCTACCCGGGTC	CCACCCCACTCCCTTCTG

Table 2.3 (Continue)

SW378	ATTATGCACCCCTACTCCCC	GATTTCTTCTTTGTTTGTGCCC
SW761	CTTTGCTCCCCATTAAGCTG	TCTAGCAAATGTCTGAGATGCC
S0038	GGGTTTTCTATTGTGTTACCATTGG	GCCTGACTTCACACTGTACTGCA
SW2008	CAGGCCAGAGTAGCGTGC	CAGTCCTCCCAAAAATAACATG
SW995	TTAAGCACTTCATGGAGCTTTG	CATAATGGAAATACCGGGTCC
SW352	GCCCCCATTCTCAATTCAC	GATCAAGCTCCCCTCTTCG
SW472	AAAATGAACCCTCTCCAGTTTC	TCTGAACACTACAGCCCGC
SW949	TGAGCAATGAGTTCAATGCC	TCGTTGGTGAAGGCATCC
S0004	GATTATGGACACGGAAGGAT	GTCCTATTTCTTGCACAGTC
S0165	GTTAACGCTTCGGGATCCTGG	GGGAGGTTGCATCTTAGATGAC
S0217	TGTGATGCAGGCTGGCAG	GCCTCCTCATCTGGGGTC
SW225	GGACCCACCAAGAGTTACC	TGCTGGTAATGGGTGATTAGG
SW1041	ATCAGAAAATGGTCAACAGTTCA	GGAGAATTCCCAAAGTTAATAGG
SW21	CGATTTTAATGTGCAGCCG	CAGGAGCTGACCTATCTGGTG
SW2404	TGACAGCCTCCTGGTTCC	AGCTGTCGTTGTTTTTCTCTCC
S0035	GGCCGTCTTATACTCTCAGCATA	CCAAATAAACAGCAGGCAGCCT
S0006	TCTGTCTGGCTTATTTCACTT	CAACCTAAGTGTCTGTCCATC
SW749	TTCCCAAACCAACCAAAGAG	AGGAACTTGCCAAAATCACG
SW2410	ATTTGCCCCCAAGGTATTTC	CAGGGTGTGGAGGGTAGAAG
SW940	TACCTCTGTGTATGCAGCACG	TGAGCATCTCATTCCGTGTC
S0295	GCCTAAAAAGACCAAAGAA	TACTGCTGAGGCAAAGGA
SW839	GGAAACCAGGATAACAGGAGG	TAACCCACTGTACCACCAAGG
SW2406	AATGTCACCTTTAAGACGTGGG	AATGCGAAACTCCTGAATTAGC
SW2419	AGGGCGTGCTCTTCTAACTG	TGACTCAGCATCTCCTGCC
SW316	TTCTCCAGCCATCATGAGTG	AATGACCATTCCTGAGGCTG
S0121	TTGTACAATCCCAGTGGAATCC	AATAGGGCATGAGGGTGTTTGA
SW322	CATTCAACCTGGAATCTGGG	TCCCTGGAAAGGCTACACC
S0385	CTATTAGGCTGGAGGGTTG	AGTTCAGAAGCTGTTGCT
SW2443	GAGCACAGAAGATTTTTAGGGC	TTAGTTTTCTCCTGGGCTGTG

2.4.2 PCR optimization

Five aspects were considered when optimizing the multiplex PCR reactions:

i) the fluorescent dye label of the primer, namely HEX, TET or FAM

ii) the fragment length or size in base pairs of the amplified DNA target material, when using similarly labeled primers in the same reaction

iii) the concentration of the primer used in the PCR reaction, noting that a lower concentration usually prevents inhibition of competing primers

iv) the average annealing temperature of the all the primers in the multiplex PCR reaction should be optimal to avoid formation of primer dimers and the appearance of artifacts (non-specific amplification)

v) allocation of primers into multiplex PCR reactions in a way that will not inhibit the operation of any primers in the PCR reaction

To determine whether all these aspects could be accommodated, each marker was first amplified separately. A stock solution of 100 pmol/µl of each primer was prepared. Serial dilutions of each stock primer were prepared, at concentrations of: 50, 25, 12.5, 6.25, 3.125 and 1.56 pmol/µl respectively. A preliminary PCR reaction was carried out (Table 2.4) and the signal strength or peak intensity was measured on an ABI Prism® 377 Automated DNA sequencer to determine the correct primer concentrations, annealing temperatures and volume of DNA for each primer set. Signal intensities of amplified fragments ranging between 500–2,000 units were selected as the optimum concentration for analysis purposes. The size of fragments in base pairs (bp) could simultaneously be determined and these values were plotted on a graph. Similarly labeled primers with overlapping fragment sizes were assigned to separate PCR multiplexes.

Table 2.4 A general reaction mixtures used for optimization of PCR reactions

Components	***Volume***	***Concentration per reaction***
Primer (serial dilution)	0.5 µl	Serial dilutions
dNTP's (2.5mM each)	0.75 µl	25 µM
10 x Supertherm Gold™ reaction buffer	1.5 µl	2 mM
DNA	1.0 µl	±20 ng
Water	4.1 µl	
Super-Therm Gold™ DNA Taq polymerase (5U/µl)	0.35 µl	2.5 U
Total	**8.2 µl**	

The final PCR protocol was optimized with different primer concentrations for each multiplex. A final volume of 8.2 µl PCR mixture contained deionized water, 25 µM

dNTP's, 0.3 mM 10 x Supertherm Gold™ reaction buffer (20 mM Tris-HCl pH 8.3, 15 mM $MgCl_2$, 50 mM KCl), Supertherm Gold™ DNA polymerase (2.5 U), different primer concentrations and 20 ng extracted genomic DNA. The amplification was performed using a Perkin-Elmer Applied Biosystems GeneAmp PCR System 9700 thermocycler (Figure 2.2). The PCR amplification reaction consisted of a 10 minute Hot Start® polymerase activation step at 95°C, 35 cycles of 45 sec denaturation at 94°C, 60 sec annealing at 60°C and 60 sec extension at 72°C; with a final extension step at 72°C for 60 minutes and a final hold of 4°C.

Figure 2.2 Perkin-Elmer Applied Biosystems GeneAmp PCR System 9700 thermocycler

The Supertherm Gold DNA polymerase enzyme is activated by heating at 95ºC for 10 minutes, which prevents excessive primer dimer formation and makes the preparation of reactions at room temperature feasible. Supertherm Gold *Taq* polymerase has the tendency to add a nucleotide (A) to the 3' end (Brownstein *et al*., 1996) of the new fragment during amplification, which may lead to problems during scoring (Zimmer and Roalson, 2005). A final extension step was included in the PCR reaction though, to ensure that this extra base was added to all the fragments (Ciofi *et al*., 1998). A porcine control, obtained from the Forensic Section at the Animal Genetics Laboratory, with known DNA profile, was included in all PCR amplification reactions, for standardization and repeatability purposes.

The multiplex PCR was considered optimized when the following criteria were met:

i) all of the alleles for the 40 microsatellite loci displayed signal intensities between 500-2000 units on the ABI 377 DNA Sequencer,

ii) no artifacts could be detected in the fragment size range within each multiplex and

iii) the profile of the porcine control sample could be determined and was repeatable.

2.5 Gel electrophoresis

Fluorescently labeled DNA fragments were seperated using polyacrylamide gel electrophoresis (PAGE), detected using a laser and analyzed using computer software. The methods used in this study is decribed below.

2.5.1 Gel preparation

Polyacrylamide gels were prepared in accordance to the manufacturer's instructions (PE Applied Biosystems 1999, Foster City, USA). Before the gel was prepared, 36-cm well-to-read plates with 0.2 mm spacers were washed with Alconox® detergent and rinsed with distilled, deionized water. DNA fragments were separated on a gel that consisted of 5% [v/v] Long Ranger® gel solution, 6 M Urea (Sigma) and 1 x TBE buffer (0.09 M Tris base, 0.09 M Boric Acid, 0.025 M Ethylenediaminetetraacetic acid, disodium salt (EDTA) pH8-Sigma). For a 36 cm gel, a 50 ml gel solution was used including 5 ml of Long Ranger solution, 10 ml of 10 X Tris Boric Ethylenediaminetetra acetic acid (TBE) buffer and 18 g urea. Deionized water (35 ml) was added and the solution gently mixed until all the urea crystals were dissolved. A 0.2 µl cellulose nitrate filter was used to filter the solution. The solution was sonicated for 5 minutes to ensure that it was homogenous. The gels were polymerised with the addition of 0.05% [v/v] N,N,N',N'-Tetramethylenediamine (TEMED) (Fluka, Neu-Ulm, Switzerland) and 0.025% [v/v] ammonium persulphate (Sigma). A sharks-tooth comb was used to form wells for sample loading. The gel was carefully poured into prepared glass plates to avoid the formation of air bubbles within the gel. Finally, the gel was allowed to polymerize for at least 2 hours.

2.5.2 Sample preparation and loading

A loading mixture was prepared containing 79 % [v/v] of deionized formamide (Applied Biosystems, Foster City, USA), 0.0072 mM GeneScan-350™ TAMRA® 350 size standard (Applied Biosystems) and EDTA/Blue dextran (3 mM EDTA, pH8, containing 2 mg/ml Blue dextran – Applied Biosystems). The PCR products were

diluted 1:35 with deionized distilled water and 1 µl of the dilute was added to 3.0 µl of loading mix. For each sample 3 µl of loading mix and 1 µl of diluted PCR product was mixed together and denatured at 95°C for 3 minutes in a thermocycler, and immediately placed on ice. A volume of 1.5 µl of the sample mix was then loaded onto the gel and electrophoresis was performed on the ABI Prism® 377 Automated DNA Sequencer (Figure 2.3 a and b). The gel was allowed to run for 2 hours before analysis.

Figure 2.3 (a) The ABI Prism® 377 Automated DNA Sequencer

Figure 2.3 (b) The ABI Prism® 377 Automated DNA Sequencer with an example of a gel image

2.6 Data collection

Fragment analysis was carried out using the ABI 377 GeneScan™ Analysis Software Version 3.1 and GenotyperII™ DNA Fragment Analysis Software Version 2.0 (Perkin-Elmer, Applied Biosystems) on an Apple™ Mac operating system Version 8.5. Before analyzing the samples, the analysis parameters were specified. The software program default options were mostly used with only minor adjustments made (Table 2.5), e.g., to the peak detection threshold (depending on the fragment height) and the analysis range (depending on the starting and ending parameters).

Table 2.5 The analysis parameters used for all samples

Peak Detection Threshold	Fam 100
	Tet 100
	Hex 100
	Tamra 80-150
Prerun Module	GS PR 36C-2,400

Table 2.5 (Continue)

Run Module	GS Run 36C-2,400
Plate Check Module	C
Analysis Range	*800-10,00
Base lined	Yes
Data Smoothing	No
Size Standard	Tamra® 350
Size call range	All
Sizing Method	Local Southern
Split Peak Correction	None

*Ranged between 800 and 1000, varying between gels

The gel image was auto-tracked using GeneScan™ (Figure 2.4). Manual examination of each lane was performed using the Tamra® 350 size standard as reference to ensure the correct assignment of each sample with regards to its position on the gel.

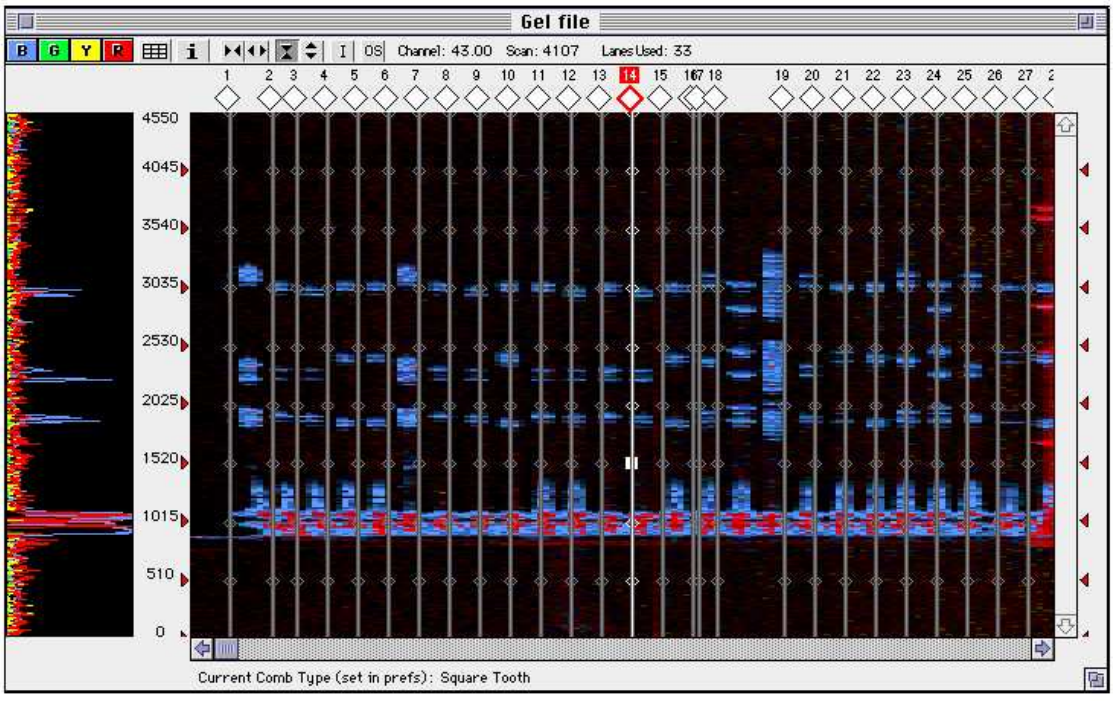

Figure 2.4 Auto-tracking of a gel to ensure the correct assignment of each sample

The Tamra® 350 standard was assigned the proper size from 35-350 (Figure 2.5) for the software to accurately calculate the correct positions of the unknown DNA fragments. A standardized gel matrix was selected during electrophoresis. The matrix

was calculated for each dye to provide a stable baseline.

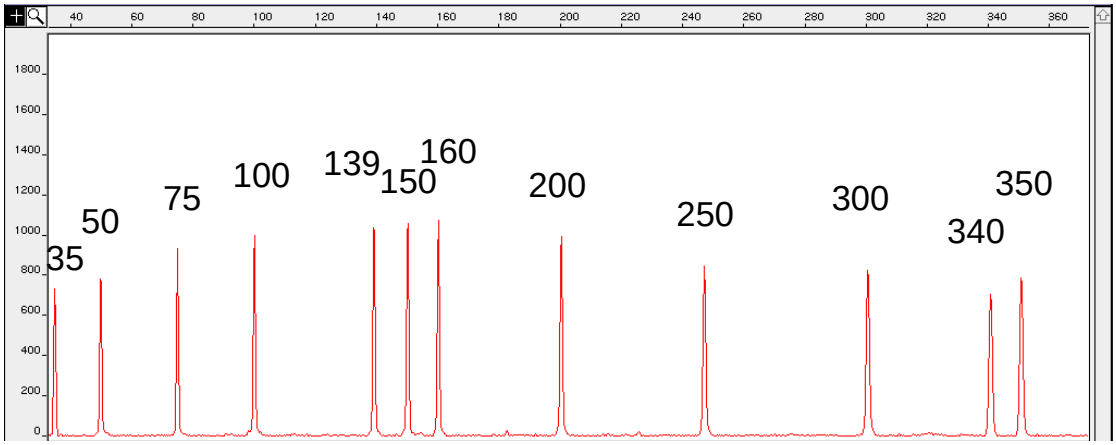

Figure 2.5 A typical designation of allele sizes for the Tamra® 350 size standard

Samples were screened using GeneScan™ and examined individually compared to the Tamra® 350 standard to prevent subsequent scoring errors. When the gel had been successfully screened, the raw data was transferred to the Genotyper™ DNA Fragment Analysis Version 2.0 for genotyping of data.

Allele classification was based on the binning of alleles compared to control samples. Allele binning is a statistical method for grouping the fragment lengths within an average peak size with a tolerance of ±0.5-0.8 into a range and allocating a specific nomenclature to it (Figure 2.6). A size range was determined for each locus, with different peak sizes appearing around actual PCR fragment lengths within the size range. A frequency histogram of all the fragment lengths in that size range was generated (Figure 2.6).

The porcine control sample was used to verify every bin during analysis of gels. Changes within the fragment lengths were manually adjusted until all allele allocations were fixed for each locus and any fragment length outside that class could be categorized as an artifact or irregularity, resulting from overloading, overflow, pull-up, contamination, electrical spike or stutter bands.

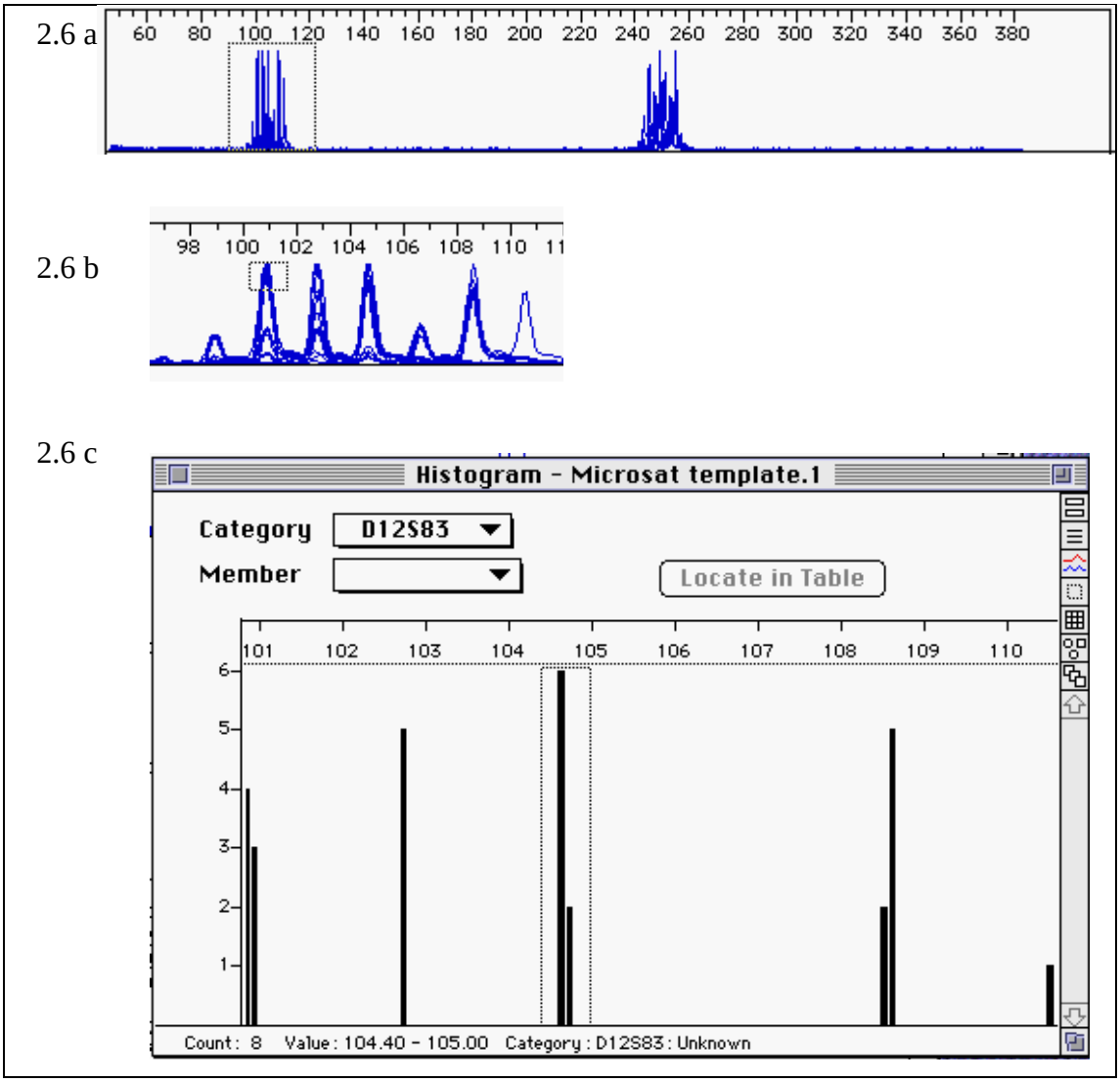

Figure 2.6 Binning of alleles: a) alleles within a range; b) enlarged view of alleles within the range; c) histograms representing the actual binning of alleles within the range

2.7 Statistical analyses

Statistical analysis of pig samples involved the use of appropriate statistical approaches and associated software. The various genetic measures and different computer programmes that were used are discussed below.

2.7.1 Organization of data

The alleles scored (fragments) for each individual at each locus were entered into Microsoft Office Excel spreadsheets. MS Toolkit (Park, 2001) was used to calculate allele frequencies and genetic diversities within breeds (see Section 2.7.4 for a full description of the latter). MS Toolkit was also used to prepare input files for some of the other software used.

2.7.2. Linkage disequilibrium (LD)

Non-random association of alleles among loci is refered to as LD (Frankham *et al*., 2002). In a population, LD, can be used to explain population history (e.g., breeding history, selection, genetic drift, mutation and admixture) (Hartl and Clark, 1997) and to map quantitative trait loci (QTL) (Andersson *et al*., 1994). Many statistical methods require that linkage disequilibrium among loci be excluded. Nevertheless, it was not considered necessary in the current study to test for possible LD among loci, since the set of pig markers used are widely applied in the Pig Genome Project.

2.8 Genetic diversity

Genetic diversity in a population reflects its evolutionary potential (Frankham *et al*., 2002). Species and populations face ever-changing environments, be it climatic changes, competitors, pollutions or diseases, and must adapt or become extinct. In artificially managed populations, genetic diversity is equally important to impart adaptability and to provide variation for artificial selection programmes. The most widely used measures of genetic diversity are various forms of heterozygosity, allelic diversity (number of alleles at a locus in the population), and proportion of polymorphic loci (Nei *et al*., 1975; Leberg, 1992). In this study, MS Toolkit was used to calculate allele frequencies and coefficients of genetic diversity.

2.8.1 Average number of alleles per locus (*An*)

Diversity was also quantified using the average number of alleles per locus (*An*) since this measure has been shown to be more sensitive to population bottlenecks than He (Spencer, 2006). MS Toolkit was used to calculate *An* values.

2.8.2 Observed heterozygosity (Ho) and expected heterozygosity (He)

The extent of genetic diversity at a locus is expressed as heterozygosity. Observed heterozygosity (Ho) was calculated as the actual number of heterozygotes at a given locus. Expected heterozygosity (He) gives an indication of the proportion of individuals that are prospective heterozygotes based on the allele frequencies and assuming Hardy-Weinberg equilibrium (Frankham, 2002). This was calculated for the microsatellite loci investigated in this study according to the formula derived by Nei (1978). Both He and Ho were calculated using MS Toolkit.

2.8.3 Hardy-Weinberg equilibrium (HWE)

In large random-mating population with no selection, mutation or migration, the allele- and genotype frequencies are expected to be constant from generation to generation (Frankham *et al.*, 2002) and is then said to be in HWE (Falconer, 1989). Testing for conformation to HWE is crucial in conservation and evolutionary genetics. It provides a basis for detecting deviations from random mating, testing for selection, modelling the effects of inbreeding and selection, and estimating the allele frequencies at loci showing dominance (Frankham *et al*., 2002). In this study, deviations from HWE were tested for using ARLEQUIN 3.1 (Excoffier *et al*., 2005). A probability test was performed using a Markov chain with 10,000 iterations.

2.8.4 Allelic Richness (*R*s)

Sample numbers per population varied greatly and for that reason a number of approaches were followed to compensate for possible errors in estimating *An*. Firstly, by using more microsatellite loci, the discriminatory ability of the marker set could be increased; secondly, microsatellites as co-dominant markers allows for an unbiased estimate of relatedness between individuals and thirdly, the allelic richness of the populations were calculated to supplement the mean number of alleles per locus.

Allelic richness is a component of genetic diversity, which takes into account the variation in sample size and should be a good indicator of past demographic changes (Toro *et al*., 2008). This measurement is especially important in conservation genetics. The observed number of alleles in a sample is highly dependant on sample size (Rodrigáñez *et al*, 2008). To bypass this problem, El Mousadik and Petit (1996) suggested adapting the rarefaction index of Hurlbert (1971) to population genetics (Petit *et al*. 1998). The principle used is to estimate the expected number of alleles in a sub-sample. The FSTAT software program (Goudet, 2001) was used to measure the allelic richness per locus and sample (*R*s), and overall samples (*R*t). It is considered that the property of allelic richness is more relevant than the property of allelic evenness in this context.

2.8.5 Rate of Inbreeding (F_{IS})

The rate of inbreeding (F_{IS}) measures the average departure from HWE in terms of the average deficit of heterozygotes within populations (Gharrett and Zhivotovsky, 2003). Values of F_{IS} in most natural populations are typically close to zero which indicates random mating within subpopulations (Hartl and Clark, 1989). FSTAT (Goudet, 2001) was used to measure the rate of inbreeding for the seven pig populations.

2.8.6 Testing for population Bottlenecks

The relationship between allelic diversity and heterozygosity was used as the basis for statistical tests to detect the presence of recent genetic bottlenecks. Cornuet and Luikart (1996) stated that populations that have experienced a recent reduction of their effective population size exhibit a correlative reduction of the allele numbers (k) and gene diversity (He, or Hardy-Weinberg heterozygosity) at polymorphic loci. The allele numbers are reduced faster than the gene diversity in such populations. To determine whether the pig population study exhibit a significant number of loci with gene diversity excess, the Wilcoxon-sign-rank test was performed using BOTTLENECK (Piry *et al*., 1999).

One of the most frequent assumptions made during calculations involving microsatellite markers is that the loci under study follow a stepwise mutation model (SMM). This model states that mutations involve the gain or loss of a single repeat, and is supported by the observation that many Microsatellites do in fact mutate in a stepwise fashion (Estoup and Angers, 1998; Ellegren, 2000a; Schlötterer, 2000). The infinite allele model (IAM) on the other hand states that mutations result in an allelic state not previously encountered in a population, and may involve any number of tandem repeats (Kimura and Crow, 1964). BOTTLENECK was used to estimate heterozygosity excess under the assumption of both the infinite alleles model IAM and SMM of mutation (with 10,000 replications in all cases).

2.9 Genetic differentiation within and between populations

Over many generations allele frequencies change due to random sampling and chance from one generation to the next, a process referred to as random genetic drift. Genetic differentiation measures the degree of genetic drift among populations (Frankham *et*

al, 2002). The rate of genetic drift is larger in small populations, and smaller in large populations. Through sampling error, genetic drift can cause populations to lose genetic variation (Pray, 2008). In this study, fixation indexes (F_{ST}, R_{ST}) and AMOVA (Excoffier, 1992) were used to demonstrate population differentiation.

2.9.1 Overall degree of genetic differentiation (F_{ST})

F-statistics, especially F_{ST}, provide important insights into the evolutionary processes that influence the structure of populations. F_{ST} measures the degree of population differentiation, and therefore genetic drift as a result of a reduction in heterozygosity, between subpopulations through the calculation of the standardized variances in allele frequencies among populations (Weir and Cockerham, 1984). F_{ST} values closer to one indicate that genetic drift between populations lead to closer genetic relationships. It is the probability that two alleles drawn randomly from a population fragment are identical by descent (Frankham *et al*., 2002). F_{ST} and associated P-values were calculated using the ARLEQUIN 3.1 (Excoffier, 2005). The sequential Bonferroni correction for multiple comparisons was applied to P-values to compensate for possible type I errors resulting from multiple pairwise comparisons (Rice, 1989).

2.9.2 Differentiation based on R_{ST} and gene flow (Nem)

Genetic differentiation using the unbiased estimator of R_{ST} is a statistical measure related to F_{ST} that was used to estimate effective migration rates since subpopulation divergence (Slatkin, 1995). The R_{ST} formula was introduced as a supplement to F_{ST} to specifically cater for the stepwise mutation model through which new microsatellite alleles are created. RSTCALC (Goodman, 1997) was used to calculate R_{ST} values and associated significance values between all population pairs. The Bonferroni correction was again applied to compensate for multiple pairwise comparisons.

Gene flow (Nem) is the transfer of genetic material from one population to another. This method is largely unaffected by variation in mutation rate or natural selection (Slatkin, 1985). Nem was used to measure the average number of effective migrants exchanged between two populations per generation, estimated from R_{ST} and F_{ST}.Gene flow values above one indicate progressively more gene flow between populations

and the migration of genetic material, whereas values below one suggest interrupted gene flow.

2.9.3 Analysis of Molecular Variance (AMOVA)

Analysis of molecular variance (Excoffier, 1992) yields estimations of genetic diversity at different levels of a specified hierarchy. AMOVA was thus used to divide overall genetic diversity among pig populations into specific hierarchical levels, using ARLEQUIN 3.1. Levels typically used are within populations, between populations or between subspecies. During the current study pig populations were grouped as follows: group 1 = established commercial breeds (SA Landrace, Large White and Duroc); group 2 = southern African indigenous breeds (Namibia, Mozambican and Kolbroek) and group 3 = outgroup (Kune-kune).

2.10 Genetic Distance

The genetic relationships between populations were also measured by determining the genetic distances among populations. One of the most widely used measures of genetic distance is Nei's (D_S) standard genetic distance (Nei, 1978). This value is proportional to evolutionary time when the effects of mutations and genetic drift are taken into consideration. However Nei *et al*., (1983) noted that the modified Cavali-Sforza and Edwards distance measure (D_A) is more efficient in determining the true topology of an evolutionary tree being constructed using allele frequency data, especially if the populations are closely related. D_A has also been reported to increase more slowly with time and maintain a linear relationship for longer periods of time (Nei *et al*., 1983). In this study genetic distance D_A between population pairs was determined using the GNKDST function of DISPAN (Ota, 1993).

2.10.1 Construction of phylogenies

Phylogenetic analysis of populations can be an important tool for studying the evolutionary relationship of populations. Though divergence times between populations are small, different genetic studies using D_A distance measures have been used on farm animal studies to determine the genetic relationships (Ruane, 1999; Brenneman *et al*., 2007; Laval *et al*., 2002; Buduram, 2004; Juodka *et al*., 2004; Zhou, 2005). It offers a simple graphic method for visualizing the relationship between the populations and bootstrap values can be added to gauge the significance

of observed patterns. The phylogenetic relationship of populations that were under investigation in this study was represented using the neighbour-joining (NJ) method in DISPAN to construct a phylogenetic tree from D_A distance measures (TREEVIEW) (Page, 1996). The NJ method tends to be less affected by the presence of admixture occurring among populations in recovering the correct topology compared with the unweighted pair-group method of averages (UPGMA) and therefore became the method of choice in this study (Ruiz-Linares, 1994; Fang *et al*., 2005). Bootstrap re-sampling (n=1,000 permutations) was performed to test the robustness of the dendrogram topologies.

2.11 Assignment of individuals to populations

Recent years have witnessed the development of assignment methods based on individual multi-locus genotypes. The Bayesian-based method is now widely used (Pritchard *et al*., 2000) to infer the number of clusters (*K*) in the data set without prior information of the number of sampling locations. This method disregard prior data on population boundaries by testing for the presence of real population structure, identifying distinct genetic populations, assigning individuals to populations and identifying migrants and admixed individuals (Pritchard *et al*., 2007). With this approach it is possible to determine the true number of genetic populations (clusters) and assign individuals probabilistically to each identified cluster. STRUCTURE (Pritchard *et al*., 2000; Falush *et al*., 2003; Kaeuffer *et al*., 2007) was used to implement a Bayesian-based assignment approach during the current study.

A genotype for each individual was entered into the input file with assumed population of origin added. The model used was based on an assumption of admixed ancestry and correlated allele frequencies. To estimate the true number of populations the parameter $\Pr(X \mid K)$ was applied, where *K* is a value of 1-11. Three independent runs for each *K* was used. All runs consisted of a burn-in period of 10,000 steps followed by 100,000 MCMC (Markov Chain Monte Carlo) iterations (Pritchard *et al*., 2000). STRUCTURE also gives the assignment probabilities of each individual for each cluster. These probabilities to infer the membership of each individual at their most probable groups.

CHAPTER 3

Results

3.1 Populations

Seven pig populations were sampled and genotyped. The following abbreviations were used to designate populations: SA Landrace (SAL), Large White (LAW), Duroc (DUR), the Namibian pigs (NAM), the Mozambican pigs (MOZ), the Kolbroek pigs (KOL) and the Kune-kune population (KK). This sequence of the seven groups was followed throughout this dissertation. The commercial populations were verified phenotypically by the AI centre officers. The indigenous pig populations were verified as authentic by the breeders whenever possible, using the breeder societies' criteria.

3.2 Samples and localities

Hair samples from the 350 individuals were collected from across southern Africa. Since the submission of samples was dependant on farmers, breeder societies and AI centre officers, small sample sizes were obtained for some of the breeds (Table 2.1). The hair samples collected had visible roots, were clean and dry.

3.3 DNA extraction

The optimum quantity of DNA for addition in the PCR reaction was determined empirically by extracting DNA from increasing numbers (2 to10) of hair roots in a set reaction mixture (see Section 2.4.2). A total of 5-9 hair samples proved sufficient for DNA extraction.

3.4 PCR optimization

Following optimization a total of 40 primers, of the initial 52 screened, were assigned to seven PCR multiplexes based on their dye label, fragment size, concentration and annealing temperature. The primers were optimized further in the respective multiplexes, by performing successive PCR reactions to adjust the annealing temperatures, concentrations, and in some cases, exchanging primers interfering with the operation of the PCR reaction. Twelve of the 52 markers were discarded (Table 3.1) due to poor amplification, interference with the PCR, the occurrence of artifacts and overlapping with other primers.

Table 3.1 Primers discarded during the PCR optimization process

Primer	Dye label	Size
SW352	Tet	100-115
S0019	Hex	200-215
SW2476	Hex	90-110
SW2067	Hex	125-142
SW445	Tet	180-210
SW1482	Tet	104-112
SW957	Hex	108-116
SW742	Hex	95-110
SWR783	Tet	175-190
S0036	Hex	114-130
SW764	Tet	110-125
S0014	Tet	198-220

The robustness of the genotyping system used was confirmed through the absence of artefacts such as allelic dropout and the amplification of multi-allelic peaks. Signal strengths of between 500–2,000 units, as measured on the ABI Prism® 377 DNA Sequencer, was used as the optimal range to determine the desirable final concentrations of primer sets (Ciofi *et al*., 1998). The size of fragments (in number of bp) could simultaneously be determined and these sizes were plotted on a graph (Figure 3.1). Similarly labeled primers with overlapping fragment sizes were assigned to separate PCR multiplexes and the final construction of the multiplexes and the associated primer concentrations are summarized in Table 3.2.

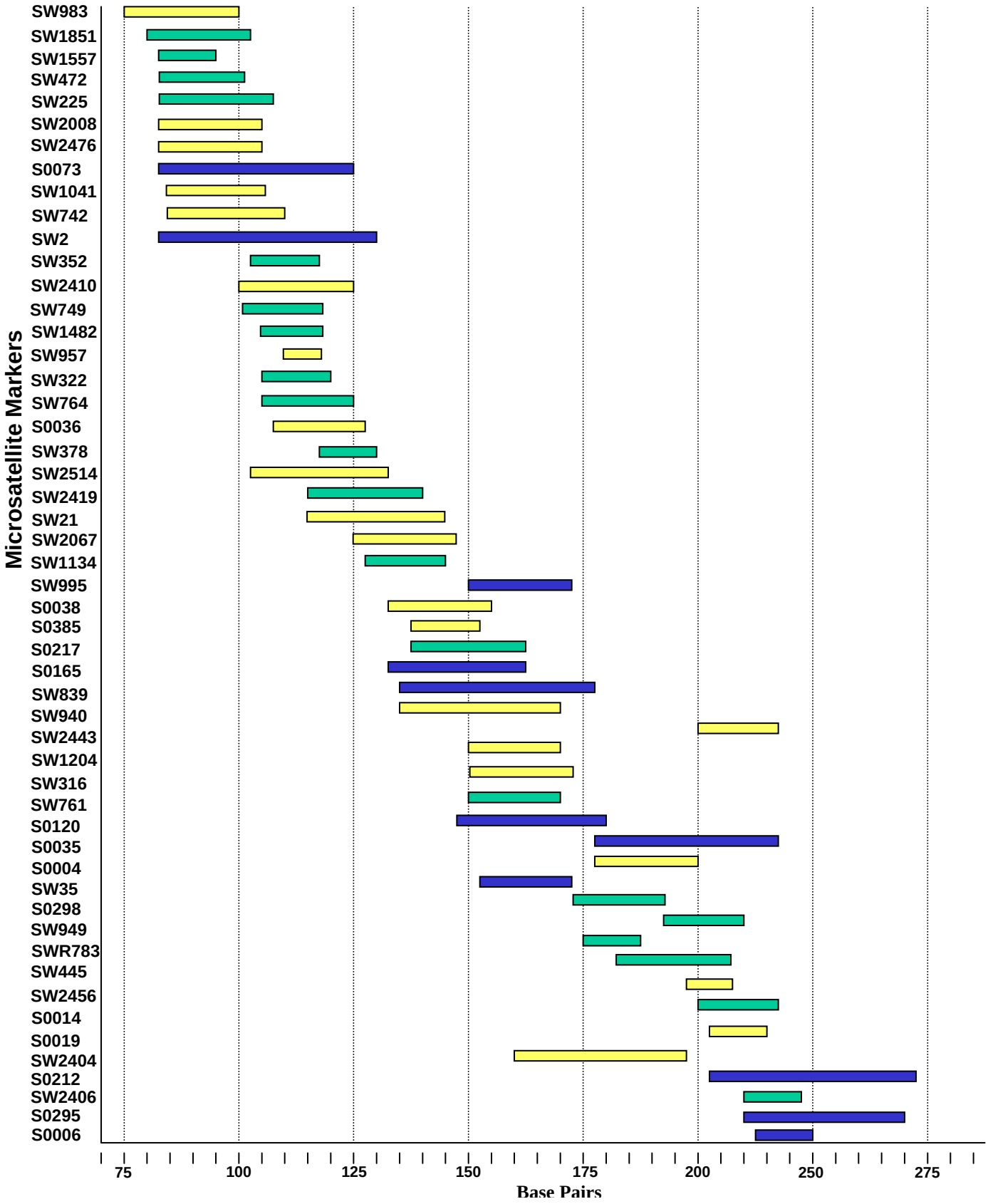

Figure 3.1 Allele sizes ranges of DNA fragments obtained for 40 microsatellite markers. The different colours correspond to the different labeled dyes used (Green = Tet, Yellow = Hex and Blue = Fam)

Table 3.2 Primers per multiplex, and primer concentrations, for seven multiplexes

Order of Loci	PLEX 1	RANGE	pMol Forward	pMol Reverse	µL/Reaction
1	S0073	90-125	5	5	0.1
2	SW35	130-170	7	7	0.14
3	S0298	170-180	5	5	0.1
4	SW1134	130-145	8	8	0.16
5	SW1851	85-105	12	12	0.24
6	SW2456	195-210	7.5	7.5	0.15
7	SW2514	105-135	12	12	0.24
8	SW983	75-100	5	5	0.1
	PLEX 2	RANGE	pMol Forward	PMol Reverse	µL/Reaction
9	S0120	145-180	6	6	0.12
10	SW2	90-130	5	5	0.1
11	SW1557	85-110	10	10	0.2
12	SW378	120-130	9	9	0.18
13	SW761	150-170	5	5	0.1
14	S0038	125-150	8	8	0.16
15	SW2008	90-110	8	8	0.16
	PLEX 3	RANGE	pMol Forward	pMol Reverse	µL/Reaction
16	SW995	150-170	6	9	0.12
17	SW352	110-115	10	10	0.2
18	SW472	88-102	6	6	0.12
19	SW949	180-215	6	6	0.12
20	S0004	160-175	8	8	0.16
	PLEX 4	RANGE	pMol Forward	pMol Reverse	µL/Reaction
21	SO165	135-170	5	5	0.1
22	SO217	140-160	4	4	0.08
23	SW225	90-115	7	7	0.14
24	SW1041	95-106	4	4	0.08
25	SW21	120-145	5	5	0.1
26	SW2404	160-190	7	7	0.14
	PLEX 5	RANGE	pMol Forward	pMol Reverse	µL/Reaction
27	S0035	160-200	6	6	0.12
28	S0006	235-250	5	5	0.1
29	SW749	102-116	6	6	0.12
30	SW2410	100-125	4	4	0.08
31	SW940	145-160	10	10	0.2
	PLEX 6	RANGE	pMol Forward	pMol Reverse	µL/Reaction
32	S0295	225-270	6	6	0.12
33	SW839	145-180	5	5	0.1
34	SW2406	220-230	7.5	7.5	0.15
35	SW2419	120-140	10	10	0.2
36	SW316	150-170	10	10	0.2
	PLEX 7	RANGE	pMol Forward	pMol Reverse	µL/Reaction
37	S0121	215-270	12	12	0.24
38	SW322	110-122	7	7	0.14
39	SW0385	140-155	8	8	0.16
40	SW2443	200-215	12	7	0.24

3.5 Statistical analysis of data

Results from the various co-efficients of diversity and differentitaion used on the pig samples, are described below. The different software programmes calculated the data and produced results that can be compared and interpreted and used to draw conclusion from.

3.5.1 Number of alleles and allele frequencies

A total of 445 alleles were detected in the 40 microsatellite loci screened (Appendix I). Locus S0295 (Figure 3.2) displayed the highest number of alleles (n=21), while SW378 presented the lowest (n=3). The average allele number (across populations) was 11.13. Amplification of the locus S038 in the Kune-kune population was unsuccessful. This locus was therefore excluded from all between population parameter calculations. Locus SW983 was found to be monomorphic in the Namibian population and Locus SW2406 in the Kune-kune pig population. Overall, the Namibian and Duroc populations exhibited the lowest number of alleles, with 157 and 159 respectively, and the Mozambican population the most with 338 alleles.

A total of 122 rare alleles were observed in 37 of the 40 loci. Of these rare alleles, 82 occurred at a frequency of less than 0.05 in 34 of the 40 loci. The threshold of 0.05 is in accordance with recommendations by Budowle (1996) and Norris *et al*. (2009). The Mozambican population exhibited 37 such rare alleles, SA Landrace 6, Large White 5, Duroc 2, Kolbroek 11 and Kune-kune 21. A total of 40 rare alleles with a frequency of more than 0.05 were recognized in 23 of the 40 loci with the Mozambican population demonstrating 8, Duroc 6, Kolbroek 5 and Kune-kune 21. Loci S0385, SW839, SW2410 and SW995 displayed the most rare alleles (n=3) of which the Kune-kune population contributed 9, Mozambique 2 and Kolbroek 1. The Namibian population did not display any rare alleles. Three loci, S0073, SW983 and SW378 did not produce any rare alleles. Overall, the Kune-kune population, considering the sample size, displayed the largest number rare alleles. No rare alleles were observed in loci S0073, SW983 and SW378.

Allelic frequencies (Appendix I) ranged from 0.003 to 0.979. The high frequency of some alleles across all populations (but usually excluding the Kune-kune population), was noticeable. This was observed at loci SW35 (137), S0298 (171), SW1134 (136),

SW983 (82), SW352 (107), SW949 (185), SW21 (128), S0006 (244), SW749 (106), SW2410 (108) and SW2406 (224).

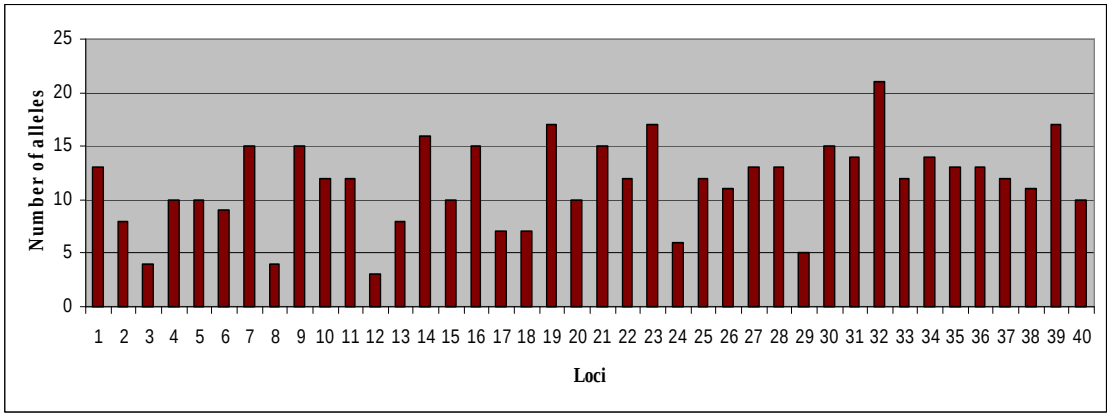

Figure 3.2 Number of alleles per locus in all populations (Locus labels 1 to 40 follow order of loci in Table 3.2)

3.5.2 Heterozygosity

The expected heterozygosity (He) estimates (Table 3.3 and Figure 3.4), ranged from 0.531 in the Duroc and Namibian populations to 0.692 for the Mozambican population. The He values of the Large white, Mozambican and Kolbroek populations were all above 0.600. The average He for the six pig populations was 0.601. The SA Landrace, Duroc and Namibian populations had values below the average He. The observed heterosygosity (Ho) estimates (Table 3.3 and Figure 3.4) ranged between 0.504 (Duroc) to 0.609 (Mozambique).

Table 3.3 Sample size, number of loci and expected- and observed heterozygosity values per population

Population	Sample size	Loci typed	Expected[1] Heterozygosity (He) (Nei, 1978)	Observed[1] Heterozygosity (Ho) (Nei, 1978)
SAL	**26**	**39**	**0.580 (0.032)**	**0.522 (0.016)**
LAW	**31**	**39**	**0.636 (0.021)**	**0.584 (0.014)**
DUR	**22**	**39**	**0.531 (0.035)**	**0.504 (0.017)**
NAM	**24**	**39**	**0.531 (0.035)**	**0.518 (0.016)**
MOZ	**166**	**39**	**0.692 (0.023)**	**0.609 (0.006)**
KOL	**61**	**39**	**0.634 (0.024)**	**0.537 (0.010)**

[1]Standard deviation indicated in parentheses

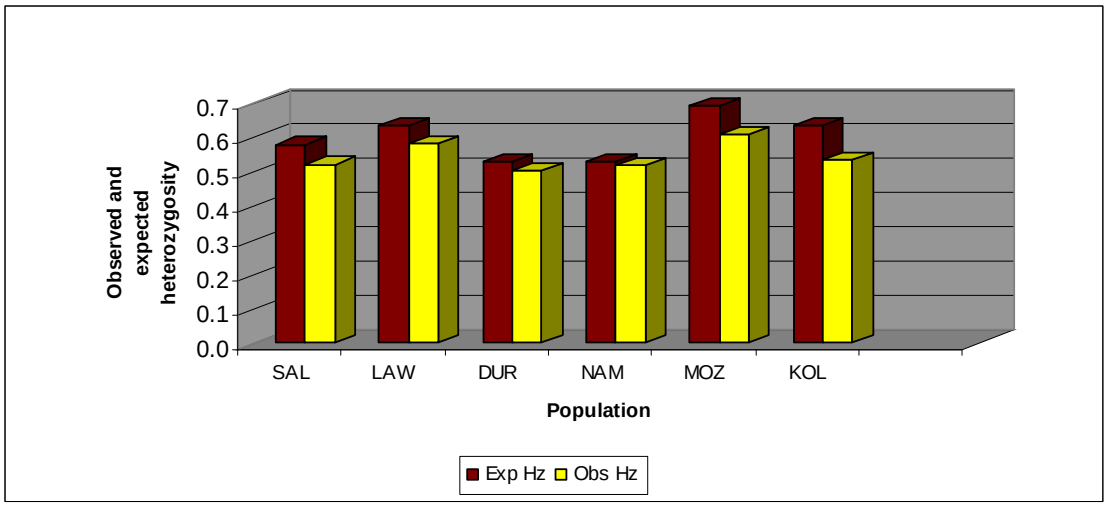

Figure 3.3 The observed- and expected- heterozygosity per population. See Materials and Methods for abbreviations used

3.5.3 Hardy-Weinberg Equilibrium (HWE)

All loci screened presented deviations from HWE except locus SW1041. A per locus analysis (see Appendix II) revealed that most loci deviated from HWE due to a heterozygote deficit ($P < 0.05$, Table 3.3), after Bonferroni corrections. The Mozambican population showed the highest proportion of deviations from HWE, with 85% of loci showing significant heterozygosity excess or deficits. The lowest number of deviations were observed in the Namibian population (12.5% of loci).

Table 3.4 Loci per pig population which showed significant deviations from expected HWE, with a significance level of $P < 0.05$ after Bonferroni correction (with * indicating significant deviations)

Locus	SAL	LAW	DUR	NAM	MOZ	KOL
S0073	*	*			*	*
SW35					*	*
S0298					*	
SW1134		*			*	*
SW1851					*	*
SW2456	*	*	*		*	
SW2514	*	*		*	*	*
SW983	*					*
S0120					*	
SW2		*			*	*
SW1557						*
SW378					*	*
SW761					*	
S038					*	*
SW2008	*					*
SW995	*			*	*	*
SW352					*	

Table 3.4 (Continue)

SW472					*	*
SW949					*	*
S0004		*	*		*	*
S0165	*	*	*	*	*	*
S0217	*	*			*	*
SW225	*	*	*			*
SW1041						
SW21					*	
SW2404	*	*		*	*	*
S0035		*			*	*
S0006			*	*	*	*
SW749					*	
SW2410					*	
SW940					*	*
S0295			*		*	
SW839					*	*
SW2406					*	*
SW2419					*	
SW316	*		*		*	
S0212	*	*			*	*
SW322					*	
S0385					*	
SW2443						*
Number	**12/40**	**12/40**	**7/40**	**5/40**	**34/40**	**26/40**
%	**30**	**30**	**17.5**	**12.5**	**85**	**65**

3.5.4 Allelic Richness (*Rs*)

In this study FSTAT was used to calculate the allelic richness based on a minimum sample size of 13 individuals per population. The results in Table 3.5 show allelic richness in each population and also compare these values to the average number of alleles calculated before. The Duroc and Namibian populations had the lowest allelic richness at 3.652 and 3.577 respectively, compared to the Mozambican population with the highest allelic richness of 5.459.

Table 3.5 Allelic richness (R_S) and number of alleles per locus (k) for all six pig populations

		SAL		LAW		DUR		NAM		MOZ		KOL	
Marker	**Allele range**	**k**	**R_S**	**k**	**R_S**	**k**	**R_S**	**k**	**R_S**	**k**	**R_S**	**k**	**R_S**
S0073	90-125	7	6.077	9	6.515	5	4.181	4	3.956	12	6.956	8	6.072
SW35	130-170	4	3.825	3	2.995	3	2.182	3	2.795	7	4.374	6	4.680
S0298	170-180	3	2.975	3	2.894	2	1.977	3	2.985	4	3.060	3	2.664
SW1134	130-145	3	2.445	6	4.317	2	1.838	2	1.542	6	4.908	7	6.047
SW1851	85-105	7	5.742	5	3.733	4	3.977	4	3.542	9	4.793	6	5.326
SW2456	195-210	5	4.244	4	3.945	5	4.913	3	2.541	7	4.165	5	4.406
SW2514	105-135	9	7.100	7	5.642	9	7.451	5	4.337	10	7.414	6	4.607
SW983	75-100	3	2.500	4	3.028	2	2.000	1	1.000	3	1.872	3	2.988
S0120	145-180	8	6.705	9	7.342	3	3.000	3	2.590	10	5.197	7	5.016
SW2	90-130	7	6.193	6	5.069	6	4.708	5	4.536	7	5.409	6	4.956
SW1557	85-110	6	5.571	7	5.595	5	4.421	4	3.909	9	5.074	11	7.310
SW378	120-130	2	2.000	3	3.000	2	2.000	2	2.000	3	2.279	3	2.817
SW761	150-170	3	2.755	4	3.779	4	3.977	3	2.911	7	4.312	3	2.212
S0038	125-150	9	7.453	8	6.215	3	2.588	6	5.586	11	7.414	10	7.647
SW2008	90-110	4	3.382	4	3.894	5	4.159	4	3.535	7	4.438	6	4.929
SW995	150-170	4	3.945	7	6.288	3	2.429	4	3.994	10	6.685	7	5.423
SW325	110-115	5	3.498	5	3.780	4	3.977	3	2.590	7	5.563	4	3.799
SW472	88-102	2	2.000	2	2.000	2	2.000	2	2.000	4	3.067	2	2.000
SW949	180-215	6	4.455	8	5.818	6	5.265	6	5.377	14	9.729	9	7.048
S0004	160-175	4	3.416	5	4.689	4	3.975	5	4.541	7	5.258	4	3.573
S0165	135-170	6	4.943	7	5.653	5	4.908	4	3.893	14	6.825	8	5.040
S0217	140-160	5	4.000	5	3.258	5	4.568	3	3.000	10	5.546	7	5.082
SW225	90-115	11	9.522	9	7.029	9	8.325	6	5.503	14	8.125	11	8.128
SW1041	95-106	3	2.882	3	2.942	3	2.591	2	2.000	4	3.055	4	3.875
SW21	120-145	4	3.637	4	3.910	6	4.611	6	4.927	9	4.679	7	5.336
SW2404	160-190	7	5.826	5	4.313	4	3.997	3	3.000	10	6.810	9	6.769

Table 3.5 (Continue)

S0035	160-200	5	4.000	5	4.618	2	2.000	4	3.911	9	5.359	4	3.313
S0006	235-250	9	7.147	5	4.989	5	4.615	9	7.716	10	7.276	5	3.590
SW749	102-116	2	1.945	3	2.667	2	1.591	2	2.000	4	2.839	2	2.000
SW2410	100-125	4	2.500	4	3.325	3	3.000	4	3.083	8	4.472	5	3.601
SW940	145-160	7	5.536	4	3.476	3	2.938	5	3.879	8	4.724	6	5.247
S0295	225-270	5	4.739	9	6.888	4	3.938	6	5.242	12	7.350	8	5.310
SW839	145-180	6	4.892	4	3.649	2	2.000	4	3.539	8	4.995	6	4.654
SW2406	220-230	3	2.877	6	4.866	3	2.182	6	5.983	14	8.467	10	6.488
SW2419	120-140	6	5.472	6	4.888	3	3.000	3	2.795	9	6.849	6	4.575
SW316	150-170	6	4.816	6	4.852	6	6.000	5	4.535	11	7.467	8	4.948
S0212	215-270	8	6.327	6	5.327	5	4.988	5	4.336	8	6.750	6	5.626
SW322	110-122	7	6.125	6	5.273	4	3.837	2	1.795	7	5.460	6	5.208
S0385	140-155	6	5.147	6	4.979	4	3.977	3	2.962	9	4.491	5	3.757
SW2443	200-215	8	6.866	8	6.276	2	2.000	3	2.706	6	4.849	8	5.826
Mean		5.475	**4.637**	5.500	**4.593**	3.975	**3.652**	3.925	**3.577**	8.450	**5.459**	6.175	**4.797**

3.5.5. Rate of Inbreeding (*F*is)

The rate of inbreeding (*F*is) estimates ranged from 0.031 (NAM) to 0.253 (KK) (Table 3.6). The lowest values were obsereved in the Namibian (0.031), Duroc (0.055) and the Large white (0.085) populations. Higher values were observed in the SA Landrace (0.106), Mozambican (0.120) and the Kolbroek (0.153) populations. The Kune-kune populations displayed a very high value of 0.253.

Table 3.6 The rate of inbreeding (F_{IS}), overall and per locus, for each pig population

LOCUS	SAL	LAW	DUR	NAM	MOZ	KOL	KK
S0073	0.396	0.350	-0.248	-0.058	0.273	0.207	0.063
SW35	0.184	0.204	-0.012	-0.224	0.216	-0.064	-0.175
S0298	0.024	-0.144	-0.077	-0.332	-0.108	0.052	-0.185
SW1134	0.364	0.661	-0.024	0	0.411	0.167	0.268
SW1851	0.037	0.246	0.065	0.138	0.191	0.310	0.115
SW2456	0.450	0.520	0.629	0.157	0.495	0.168	0.125
SW2514	0.227	0.148	-0.111	0.336	0.080	0.573	-0.127
SW983	-0.518	-0.088	-0.200	NA	0.118	0.158	0.661
S0120	-0.064	-0.054	-0.181	-0.045	0.156	0.034	0.238
SW2	0.096	0.494	0.005	0.004	-0.086	0.343	0.051
SW1557	0.250	0.199	0.108	0.071	-0.020	0.309	0.070
SW378	0.096	-0.047	0.174	-0.112	-0.164	0.117	-0.056
SW761	0.084	-0.115	0.102	-0.111	-0.053	-0.015	0.122
S038	0.237	0.152	0.192	0.167	0.120	0.095	NA
SW2008	0.012	-0.104	0.101	-0.182	-0.058	0.281	-0.061
SW995	0.298	0.160	-0.033	0.208	0.080	0.254	0.216
SW352	-0.178	0.132	0.041	0.220	0.412	0.021	0.368
SW472	0.212	0.171	0.106	0.090	0.596	0.450	0.888
SW949	-0.004	-0.085	-0.092	0.052	0.022	0.361	0.389
S0004	0.059	0.042	-0.191	-0.038	-0.057	0.116	0.346
S0165	0.783	0.700	0.175	0.673	0.504	0.465	-0.049
S0217	-0.354	-0.554	-0.085	0.009	0.068	-0.241	-0.234
SW225	0.226	0.254	-0.065	-0.037	-0.035	0.117	0.092
SW1041	0.090	-0.056	0.130	0.164	0.010	0.117	0.182
SW21	0.154	0.047	-0.145	0.050	0.003	0.013	0.143
SW2404	-0.335	-0.412	-0.035	-0.045	0.034	-0.206	-0.243
S0035	0.227	0.116	-0.162	0.280	0.244	0.028	0.546
S0006	-0.020	-0.169	-0.28	-0.060	-0.067	0.208	0.143
SW749	-0.064	0.026	0	-0.140	0.130	0.081	0.786
SW2410	-0.020	0.139	0.144	-0.088	0.093	-0.062	0.593
SW940	0.269	0.201	0.149	0.255	0.677	0.543	0.408
S0295	0.219	-0.016	0.302	0.034	0.075	-0.013	0.419
SW839	-0.179	0.235	0.045	0.008	0.176	0.180	0.631
SW2406	-0.042	0.236	-0.012	-0.096	0.053	0.229	NA

Table 3.6 (Continue)

SW2419	0.080	-0.171	-0.033	0.055	0.012	-0.092	0.430
SW316	0.282	0.104	1	-0.023	0.283	-0.002	0.478
S0212	0.022	0.140	-0.033	0.089	0.008	0.372	0.639
SW322	0.014	-0.079	-0.194	-0.022	-0.040	0.046	0.065
S0385	0.033	-0.093	-0.029	-0.288	0.085	0.090	0.237
SW2443	0.078	-0.102	0.160	-0.065	0.030	0.186	0.323
All	**0.106**	**0.085**	**0.055**	**0.031**	**0.120**	**0.153**	**0.253**

3.5.6 Testing for population bottlenecks

Screening for bottlenecks (Table 3.7) based on the IAM* showed significant heterozygosity excess in all breeds. Screening based on the SMM* showed the signature of historic bottlenecks in the SA Landrace, Large White, Mozambican and Kolbroek populations ($P < 0.05$). Very high values of 0.679 and 0.725, respectively, were obtained for the Namibian and Duroc populations during SMM screening.

Table 3.7 Signature of bottlenecks in pig populations based on the IAM and SMM models. $P < 0.05$ (in red) indicates possible historic bottlenecks

BREED	Infanite Allele Model	Stepwise Mutation Model
SAL	0.035	**0.000**
LAW	**0.000**	**0.007**
DUR	0.033	0.725
NAM	**0.000**	0.679
MOZ	**0.000**	**0.000**
KOL	**0.000**	**0.000**
KK	**0.004**	0.082

3.6 Genetic Differentiation

To determine the variation among populations, different measures were used, *i.e.*, F-statistics, gene flow, Analysis of Molecular Variance (AMOVA) and genetic distances. The results obtained using these measures are discussed below.

3.6.1 Overall degree of genetic differentiation (F_{ST})

F_{ST} values between all population pairs are presented in Table 3.8. All P-values support the hypothesis of significant ($P < 0.05$) differentiation between population pairs (after Bonferroni correction).

* see Section 2.6.4.6 for descriptions of abbreviations used

The lowest F_{ST} values were observed between the Mozambican and Kune-kune populations (0.088), and the highest values were observed between the Duroc and Namibian populations (0.263) and Duroc and Kolbroek populations (0.270), respectively. When comparing the commercial pig populations, the lowest value is between the SA Landrace and the Large White populations (0.112) and the highest value is between the SA Landrace and the Duroc populations (0.189). Among the indigenous populations, the lowest value was between the Namibian and Mozambican populations (0.123) and the highest value was between the Kolbroek and the Namibian populations (0.204). Among the commercial and indigenous populations, the lowest value was between the Large White and the Mozambican (0.128) populations and the highest value was between the Duroc and the Kolbroek (0.270) populations.

Table 3.8 F_{ST} values among the seven pig populations

	SAL	LAW	DUR	NAM	MOZ	KOL	KK
SAL	0						
LAW	0.112	0					
DUR	0.189	0.171	0				
NAM	0.184	0.197	0.263	0			
MOZ	0.135	0.128	0.181	0.123	0		
KOL	0.206	0.182	0.270	0.204	0.137	0	
KK	0.167	0.140	0.239	0.219	0.088	0.167	0

3.6.2 Differentiation based on R_{ST} and gene flow (Nem)

The trends observed for F_{ST} also apply to R_{ST} (Wright *et al*., 1978). Using the R_{ST} coefficient (Table 3.9), the values ranged from the lowest between the Kolbroek and SA Landrace populations (0.105) and the highest value between the Kune-kune and the Namibian populations (0.358). When considering the commercial pig populations, the lowest value was between the SA Landrace and the Duroc populations (0.134) and the highest value was between the Large White and the Duroc populations (0.208). Among the indigenous populations, the lowest value was between the Namibian and Mozambican populations (0) and the highest value was between the Kolbroek and the Namibian populations (0.162). Among the commercial and indigenous populations, the lowest

value was between the SA Landrace and Kolbroek (0.105) populations and the highest value was between the Large White and the Kolbroek (0.270) populations.

Pair-wise Nem values (Table 3.9) ranged from the lowest between the Namibian and the Kune-kune populations (0.448) and the highest value between the SA Landrace and the Kolbroek populations (2.132). When comparing the commercial pig populations, the lowest value was between the Large White and the Duroc populations (1.363) and the highest value was between the SA Landrace and the Duroc populations (1.619). Between the indigenous populations, the lowest value is between the Namibian and Kolbroek populations (1.290) and the highest value was between the Mozambican and the Namibian populations (∞). Among the commercial and indigenous populations, the lowest value was between the Large White and Namibian (0.953) populations and the highest value was between the SA Landrace and the Kolbroek (2.132) populations. Overall, the value of the Kolbroek population was the largest from the commercial populations (1.401-2.132).

Table 3.9 Pairwise R_{ST} values and gene flow values among the seven pig populations (R_{ST} below diagonal and Nem above diagonal)

	SAL	LAW	DUR	NAM	MOZ	KOL	KK
SAL	-	1.567	1.619	1.229	1.258	2.132	0.679
LAW	0.138	-	1.363	0.953	0.967	1.401	0.675
DUR	0.134	0.208	-	0.956	0.966	1.298	0.571
NAM	0.169	0.205	0.206	-	∞	1.290	0.448
MOZ	0.166	0.151	0.206	0	-	1.339	0.462
KOL	0.105	0.270	0.162	0.162	0.157	-	0.604
KK	0.269	0.207	0.305	0.358	0.351	0.293	-

3.6.3 Analysis of Molecular Variance (AMOVA)

In order to elucidate the partitioning of the overall genetic diversity of southern African pig populations, AMOVA analysis was applied. Results from AMOVA indicated that 82.1% of the genetic variation was caused by differences within populations, 13.8% of differentiation being partitioned among populations within designated groups (either commercial or indigenous) and 4.1% between commercial and indigenous groups (Table 3.10 and Figure 3.5).

Table 3.10 Hierarchical division of total variation in seven pig populations

Source of variation	Sum of squares	Variance components	Percentage variation
Among groups	499.326	0.630	**4.1**
Among populations within groups	786.023	2.122	**13.8**
Within populations	8534.767	12.608	**82.1**
Total	9820.116	15.360	**100**

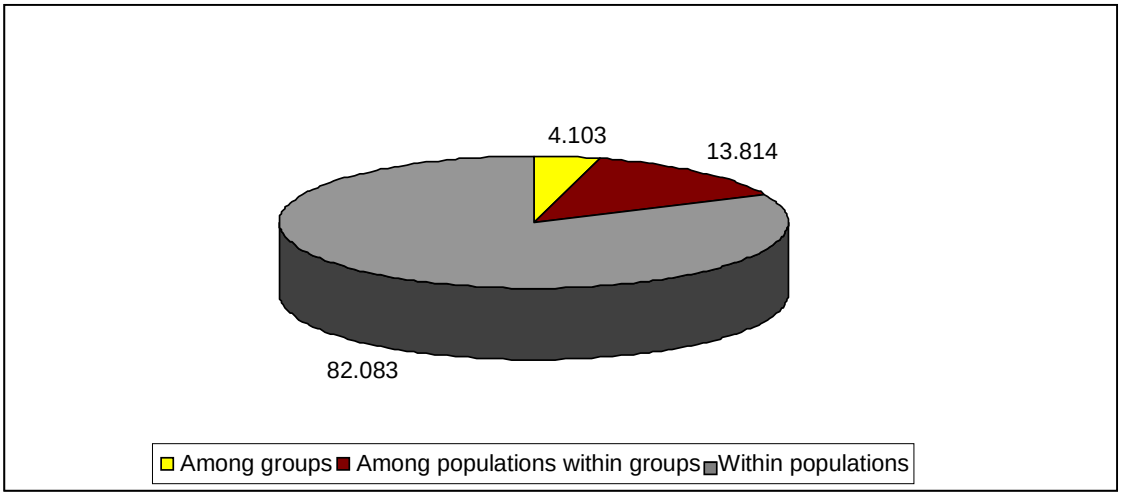

Figure 3.4 The hierarchical division of overall genetic diversity in pig populations

3.7 Genetic distance (D_A)

Nei's D_A genetic distances (1983) between each pair of seven pig populations are shown in Table 3.11. The genetic distances ranged from 0.151 (between SA Landrace and Large White) to 0.446 (between the Duroc and Kune-kune). Within the commercial pig populations, the smallest genetic distance was between the SA Landrace and the Large White populations (0.151). The biggest distance was between the SA Landrace and the Duroc populations (0.257). Within the indigenous pig populations, the smallest genetic distance was between the Namibian and Mozambican populations (0.216). The biggest distance was between the Namibian and the Kolbroek populations (0.318). Among the commercial and indigenous populations, the smallest distance was between the SA Landrace and Mozambican (0.260) and Large White and Mozambican populations (0.261). The biggest distance was between the Duroc and the Kolbroek (0.397)

populations. Overall, the distance of the Duroc population and indigenous populations was consistently high (0.361-0.446).

Table 3.11 Nei's (1983) genetic distance (D_A) values between seven pig populations

	SAL	LAW	DUR	NAM	MOZ	KOL
LAW	0.151					
DUR	0.257	0.237				
NAM	0.269	0.277	0.372			
MOZ	0.260	0.261	0.361	0.216		
KOL	0.292	0.294	0.397	0.318	0.261	
KK	0.365	0.382	0.446	0.423	0.341	0.445

A phylogenetic tree (Figure 3.5) was constructed based on Nei's D_A genetic distances (Table 3.11), to illustrate genetic distances graphically. The phylogenetic tree supports the genetic distance estimates. The dendrogram from the neighbour-joining (NJ) method (Figure 3.5) demonstrated that the Kune-kune population groups with the indigenous breeds, with a high bootstrap value of 94%. The European populations clustered together with bootstrap support of less than 50 %.

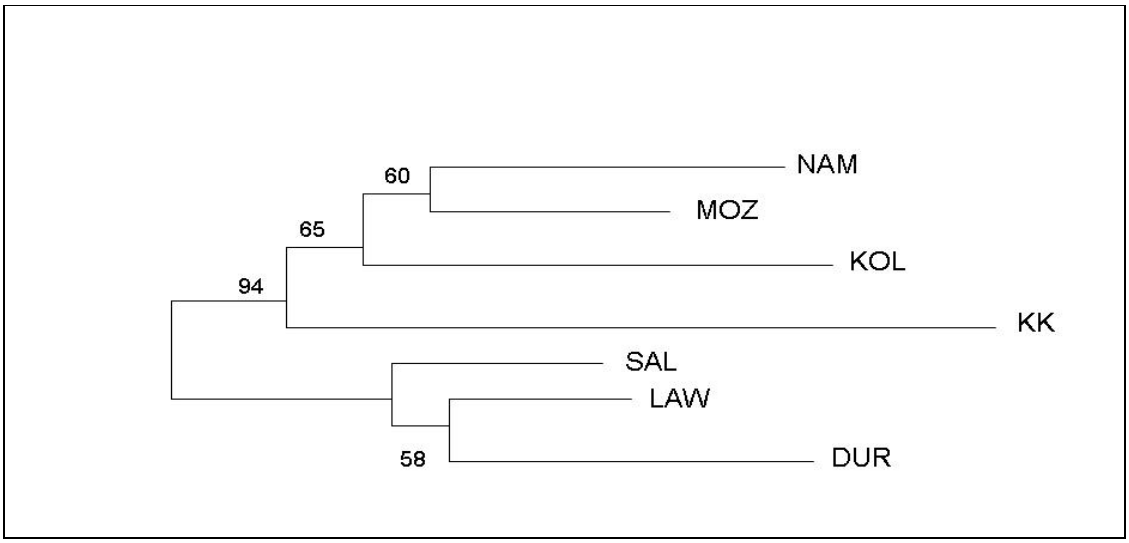

Figure 3.5 Dendrogram based on NJ method, showing the genetic relationships among seven pig breeds based on D_A genetic distance of Nei (1983). The numbers at the nodes are the percentage bootstrap values from 1 000 replications of re-sampled loci.

3.8 Individual assignment to groups

Bayesian cluster analysis performed with STRUCTURE showed that the most likely *K* value was *K* = 7. Scrutiny of values in Table 3.12, indicates that five broad genetic groups are discernable. The results showed that the commercial populations assigned to cluster 5 (SA Landrace, Large White and Duroc), Namibia to cluster 7, the Kolbroek population to cluster 3, the Kune-kune population to cluster 1 and the pigs in the Mozambican population distributed among four clusters (2, 4, 6 and 7).

The probabilities of individuals being assigned to the cluster of origin were also determined. Table 3.12 illustrates the posterior probabilities of *K* demonstrating the highest likelihood for a real structure consisting of seven populations (*K*=7). Correct assignment of samples to their population of origin was 80.8% for samples from the Kune-kune populations, 82.4% for the Kolbroek population and 98.1% for the Namibian population. The Commercial populations were assigned to one cluster with high probability, SAL 92%, LAW 96.5% and DUR 98.9%. The Mozambican populations displayed low probability of being assigned to any particular cluster. The assignment was spread over four clusters with the probability of 41.8% to cluster 6.

Table 3.12 Proportion of membership of each pre-defined population in each of seven clusters

Cluster:	**1**	**2**	**3**	**4**	**5**	**6**	**7**
SAL	0.016	0.003	0.015	0.004	**0.920**	0.006	0.036
LAW	0.006	0.002	0.004	0.003	**0.965**	0.008	0.012
DUR	0.002	0.002	0.002	0.002	**0.989**	0.001	0.002
NAM	0.001	0.002	0.003	0.002	0.007	0.004	**0.981**
MOZ	0.030	**0.219**	0.004	**0.171**	0.009	**0.418**	**0.149**
KOL	0.135	0.007	**0.824**	0.003	0.010	0.011	0.010
KK	**0.808**	0.015	0.003	0.012	0.130	0.020	0.012

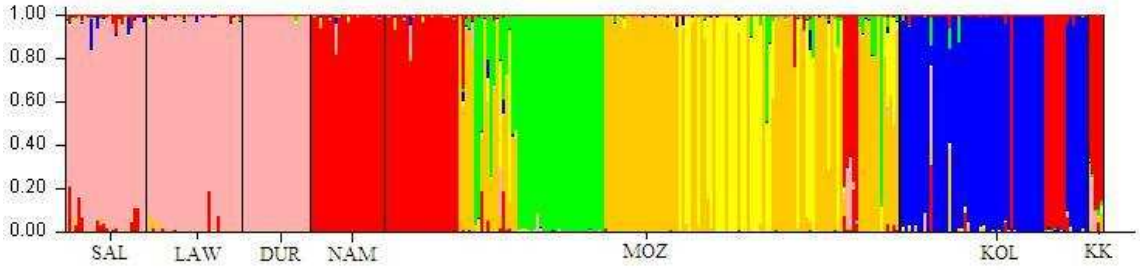

Figure 3.6 Estimated population structure based on seven pre-defined populations

In Figure 3.6, each of the 350 animals is represented by a thin vertical line that is divided into seven colored segments (*K*=7), which represent the membership of each individual to the seven clusters. The results showed that individuals nearly always shared membership coefficients in inferred clusters (Table 3.12). In several populations, individuals had partial membership in multiple clusters (Figure 3.7).

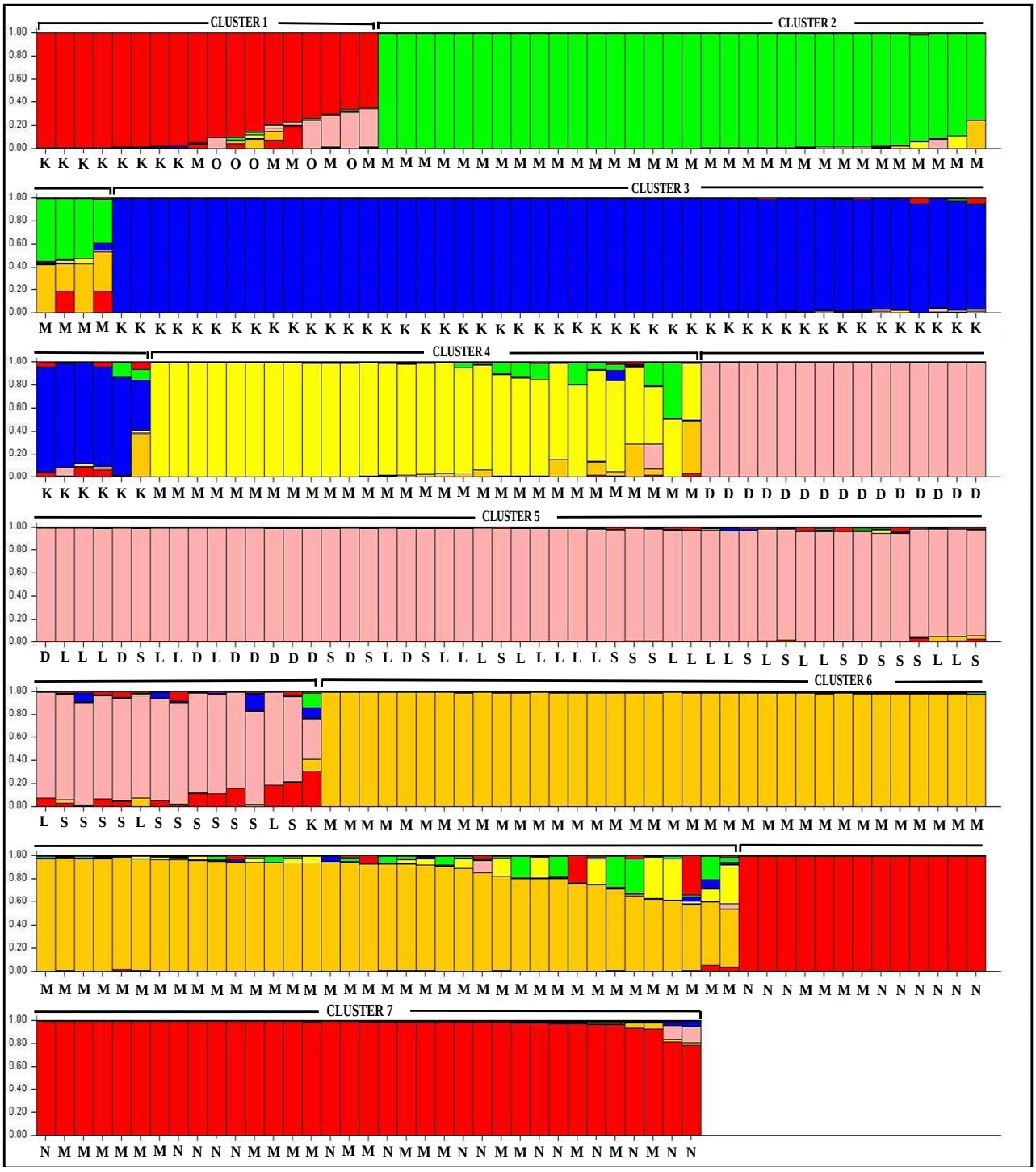

Figure 3.7 A graphical representation of the estimated population structure using the Bayesian cluster analysis of STUCTURE. Each individual is represented by a vertical bar, which is partitioned into n=*K* coloured segments that represent the individual's estimated membership fractions in *K* clusters. Populations are labelled on the X-axis and the Y-axis represents the probability of assignment of an individual to each cluster

CHAPTER 4

Discussion

4.1 Introduction

Historical information on breeding and selection strategies applied to the indigenous pig populations in southern Africa is very limited. Such information is however important since different populations potentially possess unique characteristics in terms of the quality of products, adaptation, disease resistance and hardiness. Indigenous populations could thus serve as an important reservoir of genetic diversity (FAO, 2007). From a conservation perspective, there is a need to characterize indigenous populations to maintain the diversity within populations and conserve potential unique characteristics. Knowledge of the structure of livestock populations, in terms of sources of variability among and within populations, is essential for establishing conservation priorities and strategies, with the long-term objective of maintaining genetic diversity for future generations (Notter, 1999).

The motivation for this study was to determine the genetic status of the current pig populations in southern Africa and furthermore, establish the usefulness of microsatellite markers for the characterization of pig populations. This study was therefore a first attempt to genetically characterize the indigenous pig populations despite the lack of historical information on breeding activities. It is extremely important to preserve and/or improve the existing genetic variation through breeding strategies by estimating individual breeding values and formulating breeding programmes according to the genetic information obtained.

Over the last decade, microsatellites have proved to be very useful for a range of applications. These markers were thus considered to be the marker of choice for the current study due to their large number, distribution throughout the genome, high level of polymorphism, co-dominant inheritance, neutrality with respect to selection and the ease of automation of analytical procedures (Cañón *et al*., 2001). These markers are also useful for the analysis of population structure and genetic relationships and have been widely used for genetic characterization of several pig populations, including European (Van Zeveren *et al*., 1995; Laval *et al*., 2000; Martínez *et al*., 2000; Rodrigáñez *et al*,

2008 and Lemus-Flores *et al*., 2001), Chinese (Li *et al*., 2000; Fan *et al*., 2002; Yang *et al*., 2003; Li *et al*., 2004), Mexican (Lemus-Flores *et al*., 2001) and other global pig population studies (Fan *et al*., 2005; Behl *et al*., 2006; Spencer *et al*., 2006).

4.2 Populations, localities and samples

Seven pig populations were selected to represent the southern African pig population. Sample collection for this study was dependent on submissions, with samples submitted from different populations from across the country. This method of sampling did however present the disadvantage that is was not always possible to verify that the individuals were pure and unrelated. Similarly, it was not always possible to obtain a standard sample size from each population. The minimum number aimed for was 40 individuals, but that number was not always possible and in two populations (Namibia and Kune-kune) the sample sizes were less than 40 individuals. The deficit in sample numbers was addressed by increasing the number of microsatellite markers used during this study.

The origin of the samples was limited to nucleus herds and AI stations. Sampling for the study concentrated on those sources, as the breeders and facility managers could provide information of the individuals or populations. A survey study (not published) done by Ms J. Bester, from the Agricultural Research Council at Irene, on domestic animals provided information on the location of different pig populations. It was observed that the rural pigs were scattered throughout the country and that the pigs roaming in these rural areas had little or no origin and pedigree information. These samples could therefore not be used with confidence. There are few farmers with Kolbroek populations and individuals that were submitted were representative of the Kolbroek populations in South Africa. Sampling of the other two (Namibian and Mozambican) indigenous populations was based on collections made for other studies, with the samples subsequently donated for the current study. The Namibian samples numbered less than 40 individuals, but the sampling strategy is unknown. It is recommended that sampling should be carefully approached, with a clear sampling strategy including sample size aimed for, gender composition, pedigree information and background information on localities.

A total of 350 individuals were genotyped at 40 microsatellite markers using hair samples. The advantage of using hair samples as biological material was proven during this study, since these samples proved to be a practical sample to work with in contrast to other biological samples, *i.e.*, blood or tissue samples. Hair samples were simple and inexpensive to collect, have a long storage life, easy to extract DNA from and yielded sufficient amounts of DNA from only a few hair samples (Schmitteckert *et al*., 1999).

4.3 DNA extractions

The method of extracting DNA from hair follicles, proved to be very successful. The DNA was accurately amplified using the Polymerase Chain Reaction. Sufficient DNA (with concentrations of ±2.6 ng/µl) was obtained and when stored at -4°C, the DNA could be re-used repeatedly.

4.4 PCR optimization of primers and conditions used to genotype pig populations

The results demonstrate that although multiplexing is technically challenging, when optimized it offers a cost effective, option compared to conventional single locus typing. The ease with which this technique was successfully applied to the pig samples indicates that this approach may have a broader applicability to the pig family (individual identification, forensic applications and parentage verifications). In this study, a set of 40 microsatellite markers were optimized and assembled into seven multiplexes to accommodate different dye labels and fragment sizes.

The FAO-ISAG advisory committee has recommended a panel of 27 microsatellite markers (FAO 1998; *www.toulouse.inra.fr/lgc/pig/panel.htm*) for use in domestic pigs. Many recent studies on pig populations made use of this FAO-ISAG recommended panel of microsatellites (Laval *et al*., 2000; Li *et al*., 2000; Martínez *et al*., 2000; Fan *et al*., 2002; Yang *et al*., 2003; Fan *et al*., 2005; Harcet *et al*., 2006; Chang *et al*., 2009; Lemus-Flores *et al*., 2001). The large number of studies facilitates the comparison of results between laboratories conducting pig population studies. The alternative panel of microsatellites used in this study is nevertheless still useful to quantify the genetic

diversity within and among southern African pig populations. Based on the diversity values, it can be concluded that the panel of microsatellites used for this study was suitable for the intended purpose, *i.e.*, characterization of within- and between-population genetic diversity. The results obtained from this study can now contribute to the establishment of routine DNA typing service within the ARC-Animal Improvement Institute to the advantage of the pig industry in South Africa and for forensic application with the South African Police Services.

4.5 Genetic diversity

In total, 446 alleles were identified using 40 microsatellite markers during this study. Yang *et al*. (1999) and Li *et al*. (2002) suggested that microsatellite loci, for studies of genetic diversity should have no less than four alleles per locus to reduce the standard error of distance estimates. The number of alleles in this study varied widely between loci, with as few as three at locus SW378 to as many as 21 at locus S0295. Locus SW378 was not excluded from the study though, since this level of polymorphism is comparable to results obtained during a study of Saitbekova *et al.* (1999), where the observed number of alleles ranged from 2 to 19.

In the European commercial breeds (Table 4.1), the highest average number of alleles was observed by Lemus-Flores *et al.* (2001) of 7.8. In a study of the genetic structure of Iberian pig breeds (Martinez *et al*., 2000), the average number of alleles was 4.30, whereas in a Duroc population used as reference it was 5.00. Comparable low numbers were also observed in 11 European pig breeds, with an average value of 4.59 (Laval *et al*., 2000). The lowest average number of alleles was found in the Belgian Landrace (2.9) and Large White (3.3) populations (Van Zeveren *et al*., 1995). It seems that some European and Asian pig populations have suffered a reduction in allelelic diversity. In the current study the average number of alleles in the commercial pig populations were 4.94, which compares to the values observed in some of the forementioned European pigs. The South African commercial pigs are kept under controlled mating at AI stations. The loss of diversity may be the effect of the selective breeding policy.

Compared to other studies of indigenous pigs (Table 4.1), the average allele number in Vietnamese breeds (9.95; Thuy *et a*l., 2006), Chinese breeds (7.44; Yang *et al*., 2003) and Mexican Hairless breed was significantly higher (6.8). The reasons for the higher allele numbers were that the pig populations were kept in well managed breeding programmes. The current study displayed allele numbers of 6.11 in the indigenous pig populations. The reason may be that the pigs are not under selection pressure and random mating is encouraged due to the lack of improvement programmes and the possible existence of various genetic lineages. Moderate values were observed in the Indian pig populations (5.34; Behl *et al*., 2006), Australian wild and Papua New Guinea pig breeds (5.82; Spencer *et al*., 2006) and Spanish pig breeds (5.3; Rodrigáñez *et al*., 2008). Studies on Chinese native pig breed studies revealed allele averages ranging between 4.1 and 4.4, respectively (Li *et al*., 2000; Li *et al*., 2004). These lower allele numbers in the Chinese populations indicate that isolation may have occurred. The relatively high number of alleles found in southern African populations is thus an indication that the effects of isolation and selection of these populations has been minor.

The Auckland feral populations (3.65; Fan *et al*., 2005), Chinese (2.8; Fan *et al*., 2002), Turopolje (2.4; Harcet *et al*., 2006) and Taiwan breeds (2.39; Chang *et al*., 2009) illustrated lower allele numbers. The genetic basis of the pigs in these studies was restricted and the founder effect could have had an influence on the allele diversity. The differences in variability between the southern African and global pig genotypes can possibly be explained by the choice of microsatellite markers. A total of 40 microsatelites were used in comparison to some studies using the 27 selected by the FAO and others using various microsatellite markers. The differences in polymorphism of loci can be a reason for the differences in variability with regards to other studies.

In total, a number of 122 rare alleles were observed in 37 of the 40 microsatellites analyzed. A further 82 rare alleles occurred at a lower frequency of < 0.05 and were therefore not regarded as significant. The presence of rare alleles in the microsatellite loci analyzed, mostly in the Mozambican, Kune-kune and Kolbroek populations, suggest that these loci could be useful for population differentiation and assignment, especially

because the rare alleles were often at a high frequency of > 0.05. Comparison with other studies was not possible, since none of the studies commented on the observations of rare alleles.

Heterozygosity was used as an additional method to assess genetic diversity. The range of expected heterozygosity of the markers in the six local populations in this study was between 0.531 (Duroc and Namibia) and 0.692 (Mozambique). The higher heterozygosity value in the Mozambican population may be due to the bigger sample size. By applying allelic richness measures (*R*s), the drawback of different sample sizes was addressed. In a study by Foulley and Ollivier (2006), a higher differentiation was observed in *R*s compared to gene diversity. The number of alleles is often associated with sample size, but by testing for *R*s a more exact measure is obtained for genetic diversity. Allelic richness is dependant on sample size and the heterozygosity was thus determined for 13 individuals per population. Before the application of *R*s, the average value was 8.450 and thereafter 5.459. The Mozambican population nonetheless showed a higher heterozygosity value after *R*s, but more comparable to the other populations. According to the He values, Duroc and Namibia revealed the same values of 0.531, but after *R*s, Duroc showed a higher level of heterozygosity. It is interesting to evaluate the ranking of the populations when comparing He and *R*s. For He the results (from highest to lowest) were as follows: Mozambican, Large White, Kolbroek, SA Landrace, Duroc and Namibian. For *R*s the results (from highest to lowest) were as follows: Mozambican, Kolbroek, SA Landrace, Large White, Duroc and Namibian. In the absence of accurate historical data, these trends are difficult to explain. Sample sizes obtainable during the current study could also be a contributing factor, even though the allelic richness measure compensated for sampling biases.

There is a wide variation of expected heterozygosity values among the European populations (Table 4.1). The highest values were observed by Fabuel *et al.*, 2004 (0.697); Rodrigáñez *et al.*, 2008 (0.610); Vicente *et al.*, 2008 (0.667) and Lemus-Flores *et al.*, 2001 (0.656). The average expected heterozygosity for the South African commercial populations was 0.582 which was in the range reported in European studies by Van

Zeveren *et al*., 1995 (0.590); Laval *et al*., 2000 (0.533); Martínez *et al*., 2000 (0.572) and SanCristobal *et al*., 2006 (0.560). European breeds are well-defined pure bred stock (Behl, 2006) and the same breeding principles are applied to South African commercial breeds. The lowest value was reported by Harcet (2006) in the Turpolje (Croatian) pig breeds of 0.272.

The highest average expected heterozygosity among indigenous pig populations in Asia (Table 4.1) were reported by Li *et al*., 2000 (0.719); Yang *et al*., 2003 (0.842); Li *et al*., 2004 (0.681) and Behl *et al*., 2006 (0.830). It was also higher in the Australian wild pig and Papua New Guinea study by Spencer *et al*., 2006 (0.758). The high heterozygosity levels observed during these studies may be due to low selection pressures. A study on Mexican hairless pig populations by Lemus-Flores (2001) reported an expected mean heterozygosity of 0.666. The expected heterozygosity among all the southern African indigenous populations (Table 4.1) was 0.619. Similar heterozygosity values were observed in Asian studies by Kim *et al*., 2005 (0.613) and Thuy *et al*., 2006 (0.628). Other Asian studies by Fan *et al*., 2002 (0.591) and Chang *et al*., 2009 (0.559) showed lower heterozygosity values. The lowest values were reported by Fan (2005) in the Auckland feral pig breeds of 0.450.

The Kune-kune populations displayed a heterozygosity level of 0.675. The higher value observed in the Kune-kune population could be contributed to the possibility of less selection pressures or the occurrence of population substructure (Nei, 1978). The Kune-kune population also displayed a large number of rare alleles and no signature of recent bottlenecks. This population represents a unique reservoir of genetic diversity and deserves appropriate conservation efforts.

Overall, the heterozygosity values of the different populations are closely comparable, although there is a slight difference between the commercial (0.582) and indigenous populations (0.619). The higher values in the indigenous populations could indicate that these populations tend towards random mating and low rates of selection pressure. The composition of samples used, including the possible sampling of sub-populations, could

also contribute to higher levels of heterozygosity in indigenous populations. Sample error that included possible related animals within a population should not be discarded as a possible cause.

Of the 40 microsatellite loci genotyped, 39 deviated significantly ($P < 0.05$) from HWE in one or more populations. Many factors can contribute to deviations from HWE in a population including non-random mating due to reduced population size, population subdivision ("Wahlund" effect), the presence of null alleles and a lack of neutrality relative to selection, with selection in favour of specific alleles (Maudet *et al*., 2002). It is not possible to identify the exact cause of these deviations in all populations screened. All of the pig populations in this study demonstrated significant ($P < 0.05$) deviations from Hardy-Weinberg equilibrium based on positive F_{IS} values, indicating HW equilibrium deviation in the direction of heterozygote deficit. This may be due to the "Wahlund" effect, that is, the result of the presence of subpopulations in the samples representing each group.

In the case of the Mozambican pig population, 85% of loci showed significant deviations from HWE with an F_{IS} value of 0.120. The cause may probably be fragmentation of the population into many smaller ones. Most of the Mozambican pig populations are found as small groups in remote, rural communities in isolated regions of Mozambique. In the case of the Kolbroek pig population, 65% of loci deviated significantly from HWE with an F_{IS} value of 0.153 and this could be the consequence of an original narrow genetic base and this founder effect may have been increased by the small size of the conserved population and consanguineous reproduction. Inbreeding coefficients (F_{IS}) were higher in the Mozambican and Kolbroek populations (0.153 and 0.120, respectively) than in the other populations (0.031-0.085). The Namibian population showed the least amount of deviation from HWE with 12.5% of loci deviating from HWE and it also had the lowest F_{IS} value 0.031. The Namibian samples were collected over a wide area from different locations and only a few individuals per location. The low F_{IS} could be attributed by the high genetic variation within this population and low selection pressures. Pigs kept in breeding stations and pedigree populations generally had the lowest F_{IS} values observed

during the current study, with the exception of the SA Landrace population with an F_{IS} value of 0.106. The Large White and Duroc populations showed F_{IS} values of 0.085 and 0.055 respectively. This low level of inbreeding could be due to well-planned breeding strategies.

Significant ($P < 0.05$) inbreeding was detected in the Mozambican, Kolbroek and Kune-kune populations, suggesting deviations from Hardy-Weinberg proportions might be due to consanguineous mating in some populations. The Kune-kune population presented the highest F_{IS} value of 0.253, which suggests a degree of isolation or a small founder population. These estimates indicated that the populations tend towards isolation. The results were unexpected. This was in contrast with previous values of diversity. The higher value observed in the Kune-kune population can be contributed to the possibility of a closed population or other factors, such as null alleles or occurrence of population substructure (Nei, 1978).

Compared to other pig studies (Table 4.1) the mean F_{IS} value (0.086) of SA pig populations was in the range of studies done by Korea (0.067; Kim *et al*., 2005), Portugal (0.067; Vicente *et al*., 2008), Iberia (0.045; Fabuel *et al.*, 2004) and on European Indigenous breeds (0.052; Laval *et al*., 2000 and 0.013; SanCristobal *et al*., 2006). Higher F_{IS} values were observed by Lemus-Flores *et al.* (2001) on European commercial pigs of 0.350; Fan *et al* (2005) on Aukland feral pigs of 0.168; Lemus-Flores (2001) on Mexican hairless pigs of 0.250 and Chang (2009) on Taiwanese pigs of 0.332.

According to the results, the SMM demonstrated the signature of historic bottlenecks in the SA Landrace, Large White, Mozambican and Kolbroek populations ($P < 0.05$) and is confirmed by the higher F_{IS} values. This can be attributed to either the “Wahlund” effect (the presence of sub-populations) or inbreeding of well-defined pure bred stock represented by smaller populations.

Table 4.1 A table summarizing data from all the research compared in the current study. Data summarized are the number of microsatellite markers used, average number of observed alleles, average expected heterozygosity values (He), F_{IS} values, average F_{ST} among populations and, where available, the resources

Population	Loci used	Average no. of observed alleles	Average He	F_{IS}	Average F_{ST} among populations	Resources
Southern Africa:						
Commercial	40	4.94	0.582	0.082	0.157	Current study
Indigenous	40	6.11	0.619	0.101	0.154	Current study
Pacific:						
Aukland Feral	26	3.65	0.450	0.168	-	Fan *et al*., 2005
Australian wild and Papua New Gunea	14	5.82	0.758	-	0.159	Spencer *et al*., 2006
European:						
Indigenous	18	4.59	0.510	0.052	0.270	Laval *et al*., 2000
Indigenous	50	4.5	0.560	0.013	-	SanCristobal *et al*., 2006
Commercial	10	7.8	0.656	0.350	0.110	Lemus-Flores *et al*., 2001
Belgian	7	2.95	0.590	-	0.320	Van Zeveren *et al*., 1995
Spanish	18	5.3	0.610	-	0.687	Rodrigáñez *et al*, 2008
Iberian	36	2.8-7.8	0.697	0.045	0.129	Fabuel *et al*., 2004
Iberian	25	4.37	0.572	0.059	0.130	Martínez *et al*., 2000
Turopolje	16	2.4	0.272	-	-	Harcet *et al*., 2006
Portugal	22	4.33	0.667	0.067	0.184	Vicente *et al*., 2008
Asia:						
Chinese	27	4.1	0.719	0.210	0.220	Li *et al*., 2000
Chinese	27	2.8	0.591	0.190	0.180	Fan *et al*., 2002
Chinese	26	7.44	0.842	0.274	0.077	Yang *et al*., 2003
Chinese	20	4.4	0.681	0.240	0.220	Li *et al*., 2004
Korea	16	11.6	0.613	0.067	0.261	Kim *et al*., 2005
India	23	5.34	0.830	-	-	Behl *et al*., 2006
Vietnamese	20	9.95	0.628	-	0.050	Thuy *et a*l., 2006
Taiwan	19	2.39	0.559	0.332	0.398	Chang *et al*., 2009
America:						
Mexican hairless	10	6.8	0.666	0.250	0.100	Lemus-Flores *et al*., 2001

4.6 Genetic differentiation

A hierarchiecal division of total genetic diversity indicated that 82.1% of the total genetic variation was due to differences among individuals and 17.9% was due to differentiation between populations, according to AMOVA results. Therefore, even though the majority of genetic variability was observed within breeds, there was a high population differentiation, suggesting reproductive isolation and low gene flow between some breeds. Based on F_{ST} values, the genetic differentiation observed among the populations was highly significant ($P < 0.002$) for all pairwise combinations of populations. This trend was supported by the R_{ST} calculations.

The genetic distances obtained also supported the F_{ST} values. The dendrogram (Figure 3.5) illustrates that the seven populations formed two main clusters: one composed of all the commercial populations and the other including the indigenous populations. One possible reason may be the fact that the commercial populations originated from the European pigs and that there was little influence of European populations in the indigenous pig populations. Another reason could be the historical introgression of Asian domestic pigs into European populations in the late 18th and early 19th centuries (Jones, 1998). Giuffra *et al.* (2000) found genetic evidence for introgression during that period, when investigating mitochondrial DNA variants in Asian and European pigs. This is in agreement with the results of a study using mitochondrial DNA polymorphisms from a variety of Asian and European populations as well as the Wild Boar (Kim *et al*., 2002). This tendency has been supported throughout other studies (Fan *et al*., 2002; Yang *et al*., 2003; Fan *et al*., 2005; Thuy *et al*., 2006; Megens *et al*., 2008; Chang *et al*., 2009).

A significant observation during the current study was the inclusion of the Kune-kune population into the cluster containing the indigenous populations, with 94% bootstrap support. This reflected that in almost 100% of repeats the Kune-kune population was forming a separate branch from the commercial populations. With a bootstrapping affirmation of 65%, the indigenous populations were clustered together. The results thus show a robust genetic topology not only among commercial-based populations but also among the indigenous populations.

The co-efficients of differentiation used in this study indicated that the indigenous genotypes in southern Africa have more within population variation than between population variation and clustered separately from the commercial populations.

The different measures of genetic differentiation among southern African populations will be discussed in the following sections. Measures such as F_{ST}, gene flow, genetic distances and the influence of genetic drift and/or isolation will be discussed. The genetic structure of a population at any time is the result of a balance between genetic drift and gene flow (Slatkin, 1985). The genetic distance measures will be discussed among the southern African commercial populations, indigenous populations and between all populations with reference to the aforementioned influences.

4.6.1 Differentiation among commercial populations

Among the commercial pig populations, the F_{ST} and gene flow values (in brackets) was the highest between the SA Landrace and the Duroc populations 0.189 (1.619) and between the Large White and the Duroc populations 0.171 (1.363). This indicates that the Duroc population's genetic lineage is relatively distinctive. The smallest difference was between the SA Landrace and the Large White populations 0.112 (1.567). This study showed that SA Landrace and Large White populations are genetically more similar to each other than to the Duroc pigs. This result was expected since a study done by Visser and Kotze (1996), using polymorphic markers (blood groups, allozymes and polymorphic proteins), reported that SA Landrace pigs were closer to Large White and distant from Duroc populations. This was anticipated because the Landrace breed was originally a cross between the native Danish pig and the Large White. The breed was then imported from Holland after years of selection and breeding under strict control. The Duroc population had been imported as a separate third breed. The fact that the commercial pig populations cluster together, indicates that these populations share genetic material.

Paszek *et al*., (1998), assessing linked microsatellites, found that European Landrace pigs were closer to Hampshire and distant from Duroc individuals. The current study observed, as did a previously mentioned study (Vincente *et al*., 2008 and Lemus-Flores *et*

al., 2001), that the Duroc population was significantly distant from other commercial populations. Nevertheless, the levels of genetic differentiation among South African commercial populations were rather low when it was compared with the F_{ST} values (Table 4.1) of European populations that had higher F_{ST} values ranging from 0.110 to 0.687 (Van Zeveren *et al*., 1995; Laval *et al*., 2000; Martínez *et al*., 2000; Fabuel *et al.*, 2004; Rodrigáñez *et al.*, 2008 and Lemus-Flores *et al*., 2001). These high values could to some degree be the result of selection (Neigel, 2002) in breeding facilities to maintain the typical phenotypic characteristics of the defined breeds.

The overall range of genetic distance values between the SA commercial pig populations showed closer relatedness (D=0.151 to 0.257) when compared with distances in the indigenous populations. In a study by Thuy *et al.* (2006), the genetic distances (D=0.28 to 1.93) clearly distinguished European pig breeds from Asian breeds. The European breeds studied were most related (D=0.07). Laval *et al*. (2000) concluded in a study on eleven European pig breeds that however the genetic distances (D=0.23 to 1.12) exhibited strong differentiation, it was difficult to infer reliable phylogeny. A study by SanCristobal *et al.* (2006) concluded that the commercial breeds were clustered around their breeds of reference with genetic distances of D=0.015 to 0.413. The same tendency was encountered in a study by Vicente *et al*. (2008) with genetic distances (D=0.322 to 0.680) grouping the European breeds closest together. The results from this study indicated that the commercial pig breeds in South Africa are closely related and have more within breed variation than between breed variation. The genetic distances obtained are supported by the F_{ST} estimate across all loci (0.157). The level of breed differentiation was low indicating that only 15.7 % of the total genetic variation was explained by breed differences. The SA Landrace and the Large White populations are genetically the closest related as indicated by the highest gene flow between them. This is the consequence of South African Stud breeders using F1 sows (Large White x SA Landrace) to form the pivot of the crossbreeding programmes in the majority of the commercial farms (Van der Bank *et al*., 1997).

4.6.2 Differentiation among indigenous populations

From the F_{ST} results (with gene flow values in brackets), the Kolbroek seems to be genetically more different from the other indigenous populations, especially the Namibian population 0.204 (1.339). The Kolbroek populations are mainly conserved in nucleus herds throughout the country. Farid *et al.* (1999) observed complex patterns of gene flow in most British sheep breeds and suggested that these breeds may have been kept in isolation for extended periods. Similar mechanisms may be at work in local populations, and it is thus possible that the Kolbroek population could have been subjected to isolation for long periods. The values between the Namibian and Mozambican populations F_{ST}=0.123 (∞) indicate closer identity. The average F_{ST} value among the indigenous pig populations was 0.154.

F_{ST} values observed in studies by other authors (Table 4.1) among other indigenous pig breeds, varied from 0.050 to 0.398. Similar results to the current study was observed in studies by Spencer *et al.* (2006) with F_{ST}=0.159 and Fan *et al.* (2002) with F_{ST}=0.18. Lower F_{ST} values were observed in studies by Yang *et al.* (2003) with F_{ST}=0.077 and Thuy *et al.* (2006) with F_{ST}=0.050. Conversely, higher F_{ST} values were observed in Asian studies by Li *et al.* (2000) with F_{ST}=0.220; Li *et al.* (2004) with F_{ST}=0.220; Kim *et al.* (2005) with F_{ST}=0.261 and Chang *et al.* (2009) with F_{ST}=0.398. The values obtained in these studies were attributed to adaptation to specific circumstances.

Genetic distance values ranged from D=0.216 to 0.318 in the indigenous populations. Genetic distances and the dendrogram confirmed that among the indigenous populations the Kolbroek population is genetically more distant (D=0.318) from the Namibian and the Mozambican populations (D=0.261). The distant relationship between the Kolbroek and Namibian populations indicated that geographic barriers separated these populations. Although phenotypically similar, the populations have less in common genetically. The Namibian and Mozambican populations are also genetically close (D=0.216), which is unexpected due to the geographical distance and the phenotypic characteristics. The genetic differentiation among indigenous pig populations could originate from several factors. It could be associated with the introduction of Asian pig populations used as

barter along the coastline of southern Africa. Also, it may originate from the distant contribution of founder populations, the different degree of crossbreeding, and selection imposed by selective factors such as adaptation to different ecosystems and economic and social pressures.

Compared to other studies on indigenous populations, the southern African indigenous populations were closer related. The only study that yielded results comparable to the southern African populations was a study done by Fan *et al.* (2002) of which the genetic distances (D=0.128 to 0.231) demonstrated that the Chinese breeds are genetically closer. In other studies the genetic distances indicated greater distances between the populations. A study by Li *et al.* (2000), for example, indicated that the Chinese breeds clustered together and the genetic relationship (D=0.475 to 1.047) was consistent with their geographical position. The genetic distance (D=0.177 to 0.672) in a study by Kim *et al.* (2005) showed that the Chinese breeds are closely related but the Korean pig breed have been crossbred with a European pig population. Another study by Fan *et al.* (2003) on Chinese pig breeds concluded that genetic distance (D=0.255 to 0.516) showed that the relationship between these pigs were a result of their similar geographical distribution. Genetic distances (D=0.025 to 0.264) in a study by Chang *et al.* (2009) showed that the Asian pig breeds are closer related although the results suggested minimal gene flow between these breeds in recent times.

4.6.3 Differentiation among all populations

The F_{ST} (with gene flow values in brackets) values were the highest 0.270 (1.298) between the Kolbroek and Duroc populations and the Namibian and Duroc populations of 0.263 (0.956). This could be explained by the isolation of the indigenous populations from the commercial populations. Values of 0.181 (0.966) were observed between the Mozambican and Duroc populations. The lowest values of 0.128 (0.967) were observed between the Mozambican and Large White populations and between the Mozambican and SA Landrace populations of 0.135 (0.967). This result was unexpected. It can only be assumed that there was some introgression from the commercial pigs into indigenous populations in Mozambique. Values of 0.197 (0.953) were observed between the

Namibian and Large White populations and 0.184 (1.229) between the Namibian and SA Landrace populations. Between the Kolbroek and Large White and Kolbroek and SA Landrace populations, values of 0.182 (1.401) and 0.206 (2.132) were observed respectively. The 2.1 migrants per generation observed in this study leads to similar conclusions compared to the genetic distance values. This can possibly indicate that the SA Landrace served as a mothering line (F1 sow) to the Kolbroek population. There is however no breeding or historical information to base these conclusions on.

The Kune-kune population proved to be a good choice as reference group according to the results. The highest values were between the Kune-kune and Duroc populations of 0.239 (0.604). The lowest value was observed between the Kune-kune and Mozambican populations of 0.088 (0.462). In a study by Gongora *et al.* (2002), the maximum likelihood analysis and the NJ tree showed the Kune-kune clustered with Asian domestic pigs that are supported by the results in this study. The indigenous populations in southern Africa may have experienced introduction of Asian swine genetic material. The Kune-kune population showed significant differentiation from all the other populations (D=0.341 to 0.446). Genetically, the Kune-kune is distinct from the Duroc (D=0.446) and Kolbroek (D=0.445) populations. Between the Kune-kune and Mozambican population, the genetic distance is the lowest (D=0.341). As mentioned before, this result can be attributed to the influence of the Asian pigs on both the Kune-kune and Mozambican populations. The large genetic distances among the indigenous and commercial pig populations and the different evolutionary paths taken by these populations could suggest that the indigenous pig populations belong to a different and more ancient genetic lineage, distinct from the one giving rise to the modern commercial pig populations. This different lineage of genetic differentiation is consistent with the high F_{ST} values.

4.6.4 Individual assignment

The individuals of the populations were assigned and classified to known populations of which the likelihood of their genotypes was highest. Following the determination of seven as the true value of *K*, the proportional contribution of the assumed base

populations to each one of the current populations was computed and the corresponding results confirmed that each one of the populations was closely identified with one of the putative populations. Progressively, as *K* increased, the contributions of the assumed populations resulted in the separation of the seven populations, which were essentially identified with each one of the nominal populations when *K*=7. When the principle of assigning an individual to the population with the major contribution to its genome was used, a large portion of the animals were correctly classified in their original population. This result is in line with the F_{ST} estimate and confirms the distinctiveness and low gene flow between the pig populations analyzed. Interestingly, the Mozambican population, showed the largest mixture of the base population. It can be concluded that animals in this population is representative of the Mozambican population and classified with all the other indigenous populations. Subpopulations may be evident as demonstrated before with the "Wahlund" effect. The commercial pig populations did not separate into different clusters, which illustrate the close genetic relationship between the commercial populations and that their base population must be very similar.

4.7 Summary of trends

The indigenous populations have the greatest levels of heterozygosity and numbers of alleles indicating that the indigenous populations represent a potential reservoir of genetic diversity for future selection that deserves appropriate conservation efforts. However, a deficit in heterozygosity was observed in the Kolbroek and Namibian populations. The danger of genetic erosion should be considered for the Kolbroek and Namibian populations where a degree of inbreeding was observed and serious conservation strategies must be applied.

The results suggest further that the populations studied were genetically closer than expected. Deviations from HWE indicate a limited effect due to artificial selection that occurred in many of the populations particularly the Namibian and Kolbroek populations.

The results also yielded evidence that the Kune-kune population can be considered to be an independent population. Furthermore, loci with rare alleles for the Mozambican,

Kolbroek and Kune-kune populations, were observed. The results demonstrate that the indigenous populations harbour a valuable reservoir of uniqueness and can support livestock bio-conservation activities.

There is considerable genetic variation within the populations, which implies that great potential exists for improvement of the Kolbroek (a breed already recognized) population through selection. The average genetic distance between the indigenous populations is very small and the three indigenous pig populations clustered together, but are clearly separate populations. To obtain accurate information on genetic variability between pig populations or populations an average sample size of 40 animals per population is recommended for population genetic characterization. Therefore, further investigation using bigger sample sizes of each population is advised.

It was evident from the results that little genetic differentiation exists between the three indigenous populations. The large genetic distance between the Kolbroek and SA Landrace was expected. These two populations should be conserved as they are genetically distinct populations.

CHAPTER 5

Conclusions and Recommendations

The specific aims set for the current study were achieved as follows:

i) A panel of 40 microsatellites were optimized and validated and were suitable for the intended purpose of genetic characterization and breed differentiation. The panel of markers exhibited high polymorphism and there are strong indications that null alleles occurred at a low frequency.

ii) It was possible to describe genetic diversity and differentiation and establish a clear genetic structuring among the three principal commercial pig breeds (the Landrace, the Large White and the Duroc) and the indigenous pig breeds (the Kolbroek, Mozambican and Namibian) of southern Africa using this panel of microsatellite markers.

The most significant outcomes of this study were as follows: firstly, the set of microsatellite markers used in this study was generally suitable in evaluating genetic diversity in the pig populations analyzed, revealing moderate to high levels of genetic variability, demonstrated by both the number of alleles and heterozygosity. Furthermore, the pig populations demonstrated moderate levels of differentiation. Some of the populations showed signs of inbreeding (Kune-kune) and others (SA Landrace, Large White, Mozambican and Kolbroek) have gone through genetic bottlenecks in the recent past. Finally, the indigenous pig populations clustered together and demonstrated more genetic distinctness when compared to the commercial populations. Care should be taken in future conservation strategies to preserve the uniqueness of these populations. The large genetic distance between the Duroc and SA Landrace indicates separate development, while the close similarity of the Namibian and Mozambican populations indicated high gene flow and common ancestors. A more extensive study may be required to solve this question.

This study represents the first attempt to genetically characterize certain pig populations in southern Africa. It provides new knowledge for the preservation and utilization of genetic resources obtained from the analysis of relationships among different pig

populations. Furthermore, this is an important contribution to the pig-breeding sector since assessing genetic diversity should be the first step in establishing appropriate management strategies for any livestock species (Vicente *et al*., 2008). Factors leading to the loss of genetic diversity must be identified through continual genetic evaluation and appropriate management strategies implemented when necessary.

The results reported in this study will serve as useful indicators in setting breeding and conservation priorities, based on both among-population diversity and within-population variability. The results could in future be applied to linkage studies together with additional information on traits of potential economic importance, including adaptation, disease resistance and desirable phenotypic characteristics. Monitoring of gene flow among populations will facilitate determination of levels of inbreeding and cross-breeding. Breeders of indigenous pigs should apply a management strategy in collaboration with the ARC Animal Improvement Institute, recording pedigree information, mortalities, fertility, and phenotypic characteristics. The breeders should then manage reproductive programmes to avoid further loss of diversity and the occurrence of deformities and loss of production potential (as possible manifestations of inbreeding). The use of available expertise existing at the ARC and with private consultants should be encouraged.

The future impact of this study for the pig industry will be to contribute to molecular data to the management decisions made in this industry. Specifically, this will be based on a robust marker set that can be applied for (i) individual identification (DNA profiling); (ii) breed characterization; (iii) paternity testing and (v) forensic applications. Methods for genotyping were optimized during the current study and genotyping can now be applied as a routine laboratory procedure. Furthermore, individuals from the indigenous populations have been identified as having rare alleles and this may be an indication of high overall diversity within these populations that may provide diversity for future selection programmes.

The results of this study also highlighted the need for further studies to refine our knowledge of the relationships among the commercially important pig populations and the indigenous pigs, and the need to assist in genetic characterization and future conservation. Data on specific traits of economic importance, special adaptive features and the importance of breeds locally and culturally should be considered during the formulation of management and conservation decisions.

Although the markers used in this study were suitable to determine the genetic diversity and differentiation of the pig populations in southern Africa, it is recommended that the panel recommended by the FAO-ISAG advisory committee should be applied in future studies of SA pig populations, in order to facilitate comparison of results between laboratories. Furthermore, the sample size used for different pig populations should be standardized during future studies to eliminate possible erroneous conclusions based on sampling error. Sample sizes should ideally also be increased, for results to be fully representative of the different populations. A suitable sampling strategy must be formulated collecting at least 40 unrelated animals (30 females and 10 males) per population. Samples must be personally collected and recorded by researchers, with less dependence on third-party submissions. Finally, the database based on STR technique can be supplemented by using additional markers such as SNPs and analysed approaches based on QTLs. A future database should thus provide comprehensive data on traits of economic importance, data related to technology in disease resistance and the identification of loci important to animal health, efficient production, germplasm selection and gene mutations.

CHAPTER 6

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APPENDIX I: NUMBER OF ALLELES AND ALLELIC FREQUENCIES

Number of alleles and allelic frequencies for 40 loci per population. Rare alleles with a frequency of < 0.05 is highlighted in red and rare alleles with a frequency > 0.05 is highlighted in blue

S0073	SAL	LAW	DUR	Nam	Moz	Kol	KK
96	0.135			0.333	0.091		
102	0.038	0.016					
104					**0.012**		
106	0.346	0.177			0.045	0.033	
108			0.318		0.003	0.115	
110	0.019	0.032			0.055	0.008	0.167
112	0.327	0.516	0.477	0.458	0.427	0.221	0.750
114	0.077	0.016	0.159		0.009	0.426	
116		0.016			0.030	0.025	
118	0.058	0.113	0.023	0.083	0.245		
120		0.081	0.023		0.055	0.123	0.083
122		0.032			0.009	0.049	
124				0.125	0.018		

SW35	SAL	LAW	DUR	Nam	Moz	Kol	KK
127						**0.008**	
129					0.006		0.083
131	0.154				0.048	0.025	0.028
133					0.006		0.028
135	0.058	0.258	0.023	0.292	0.214	0.336	0.444
137	0.712	0.597	0.955	0.667	0.392	0.123	0.083
139	0.077	0.145	0.023	0.042	0.319	0.115	0.306
141					0.015	0.393	0.028

S0298	SAL	LAW	DUR	Nam	Moz	Kol	KK
171	0.596	0.435	0.909	0.479	0.436	0.049	0.824
173	0.096	0.500	0.091	0.104	0.424	0.877	0.176
175	0.308	0.065		0.417	0.136	0.074	
177					**0.003**		

SW1134	SAL	LAW	DUR	Nam	Moz	Kol	KK
134		0.016	0.045		0.154	0.254	
136	0.904	0.597	0.955	0.979	0.518	0.156	0.088
138	0.077	0.290		0.021	0.081	0.254	0.324

140		0.032			0.123	0.033	0.588
142					**0.120**		
144					**0.003**		
150	0.019	0.016				0.082	
154						**0.025**	
156						**0.197**	
158		**0.048**					

SW1851	SAL	LAW	DUR	Nam	Moz	Kol	KK
80	**0.019**						
82					**0.003**		
86	0.058				0.009	0.049	
88	0.058	0.065	0.318	0.438	0.301	0.049	0.306
90	0.115			0.292	0.105	0.107	
92	0.019	0.016	0.091	0.021	0.018	0.484	0.028
94	0.615	0.468	0.250	0.250	0.298	0.254	0.528
96	0.115	0.435	0.341		0.259	0.057	0.139
98		0.016			0.003		
100					**0.003**		

SW2456	SAL	LAW	DUR	Nam	Moz	Kol	KK
187	0.019					0.115	0.031
189	0.538	0.083	0.091		0.078	0.262	0.094
201			**0.136**				
203					**0.003**		
205	0.288	0.200	0.273	0.188	0.184	0.082	0.688
207	0.115	0.567	0.432	0.792	0.602	0.516	
209	0.038	0.150	0.068	0.021	0.123		
211					0.006	0.025	0.188
213					**0.003**		

SW2514	SAL	LAW	DUR	Nam	Moz	Kol	KK
100						**0.009**	
106	0.096	0.032	0.045			0.093	
108	0.058						0.139
110	0.462	0.516	0.045	0.458	0.256	0.176	0.056
112	0.115	0.065	0.023	0.021	0.039	0.231	
114	0.038		0.068		0.154	0.019	0.167
116					0.018		0.139

118	0.019	0.145	0.614	0.208	0.151	0.472	
120	0.019	0.194	0.045		0.117		0.250
122	0.173			0.271	0.139		0.222
124			0.045		0.105		0.028
126		**0.016**					
128	0.019		0.023		0.015		
130				0.042	0.006		
132		0.032	0.091				

SW983	SAL	LAW	DUR	Nam	Moz	Kol	KK
82	0.635	0.871	0.818	**1.000**	0.958	0.59	0.925
84	0.019	0.032				0.139	0.050
88		0.016			0.024		
92	0.346	0.081	0.182		0.018	0.270	0.025

S0120	SAL	LAW	DUR	Nam	Moz	Kol	KK
141					**0.003**		
147							**0.024**
151	0.038	0.113			0.003		
153				0.042	0.158	0.008	
155		0.129			0.042		0.119
157	0.077	0.065			0.003	0.042	0.310
159	0.135	0.016			0.021		0.119
161	0.038					0.110	0.024
163	0.038	0.274					
165	0.019	0.097	0.636		0.003	0.025	0.024
167	0.404	0.258	0.182			0.093	
169	0.250	0.032	0.182	0.917	0.412	0.686	0.286
171				0.042	0.303	0.034	0.048
173					0.052		0.048
175		**0.016**					

SW2	SAL	LAW	DUR	Nam	Moz	Kol	KK
91						**0.051**	
99	0.096	0.016	0.068		0.018		
101	0.019	0.032		0.250	0.328	0.246	0.333
103					**0.232**		
105	0.096					0.161	
107	0.115	0.306	0.136	0.021	0.024	0.008	

109	0.404	0.306		0.125	0.054	0.102	0.167
111		0.226	0.727	0.333	0.292		0.214
113	0.038	0.113		0.271	0.051	0.432	0.286
115	0.231		0.023				
119			**0.023**				
121			**0.023**				

SW1557	SAL	LAW	DUR	Nam	Moz	Kol	KK
83					0.003	0.042	0.026
85					0.114	0.008	0.079
87	0.115	0.177	0.045		0.006	0.068	0.605
89	0.250	0.097	0.364	0.063	0.012	0.017	
91	0.058	0.065	0.023			0.407	0.079
93	0.462	0.565	0.455	0.438	0.286	0.254	0.105
95	0.077	0.016	0.114	0.354	0.259	0.051	0.053
97	0.038	0.016		0.146	0.289	0.059	0.026
99		0.065			0.027	0.068	0.026
101						**0.017**	
103					**0.003**		
109						**0.008**	

SW378	SAL	LAW	DUR	Nam	Moz	Kol	KK
122		0.210			0.012	0.058	
124	0.750	0.452	0.750	0.396	0.584	0.142	0.925
126	0.250	0.339	0.250	0.604	0.404	0.800	0.075

SW761	SAL	LAW	DUR	Nam	Moz	Kol	KK
148					**0.009**		
150					0.322	0.009	
152					**0.033**		
156	0.038	0.226	0.227		0.012		0.211
158	0.231	0.629	0.250	0.229	0.262	0.853	0.263
160	0.731	0.097	0.091	0.708	0.352		0.421
162		0.048	0.432				0.105
164				0.063	0.009	0.138	

S038	SAL	LAW	DUR	Nam	Moz	Kol	KK
126					**0.003**		
130					0.080	0.121	

132	0.313	0.224	0.841	0.250	0.188	0.103	
134			0.023				
138	0.104				0.016	0.181	
140	0.188	0.086					
142	0.021	0.017		0.227	0.032	0.164	
144	0.229	0.379		0.136	0.054	0.035	
146		0.190		0.023	0.035	0.129	
148	0.021				0.061	0.009	
150			0.136	0.227	0.344	0.216	
152	0.042	0.069		0.136	0.175		
154	0.042	0.017			0.013		
158						**0.035**	
162	0.042	0.017					
164						**0.009**	

SW2008	SAL	LAW	DUR	Nam	Moz	Kol	KK
90			0.023		0.009		
92						**0.034**	
94	0.019				0.037		
96		0.065		0.022	0.177	0.186	
98					0.003	0.017	
100	0.654	0.323	0.682	0.696	0.668	0.119	0.071
102	0.269	0.387	0.182	0.196	0.068	0.559	0.714
104	0.058	0.226	0.023	0.087	0.037	0.085	
106							**0.214**
108			**0.091**				

SW995	SAL	LAW	DUR	Nam	Moz	Kol	KK
139						**0.034**	
141						**0.051**	
143		0.032			0.030		
147					**0.340**		
149							**0.050**
151						0.051	0.050
153	0.327	0.274	0.023	0.354	0.364	0.356	0.175
155	0.077	0.145	0.932	0.125	0.072	0.246	
157	0.212	0.032	0.045	0.313	0.045	0.254	0.050
159	0.385	0.129			0.012	0.008	0.325
161		0.290			0.021		0.200

163		0.097		0.208	0.042		0.100
165							**0.050**
167					**0.015**		
169					**0.057**		

SW352	SAL	LAW	DUR	Nam	Moz	Kol	KK
101					**0.003**		
105			0.182	0.042	0.253		0.375
107	0.788	0.355	0.091	0.917	0.446	0.631	0.250
109	0.019	0.016	0.364		0.063	0.057	
111	0.154	0.532	0.364	0.042	0.093	0.123	0.375
113	0.019	0.081			0.072		
115	0.019	0.016			0.069	0.189	

SW472	SAL	LAW	DUR	Nam	Moz	Kol	KK
86							**0.273**
88							**0.273**
90							**0.045**
92					0.152		0.091
94	0.788	0.645	0.455	0.438	0.585	0.770	0.318
96	0.212	0.355	0.545	0.563	0.261	0.230	
100					**0.003**		

SW949	SAL	LAW	DUR	Nam	Moz	Kol	KK
163					0.015	0.017	
165					**0.042**		
167					**0.060**		
171					0.009	0.017	
173			0.136		0.090		0.026
175					0.136		0.079
183	0.038			0.063	0.021	0.271	0.026
185	0.808	0.355	0.409	0.542	0.22	0.254	0.158
187		0.016			0.036	0.059	0.158
189	0.019	0.016		0.083	0.157		0.053
193		**0.016**					
197							**0.026**
199	0.019					0.085	0.026
201	0.038	0.065	0.045	0.208	0.06	0.144	0.158
203		0.032	0.023	0.021	0.069	0.025	

204					**0.006**		
205		0.177	0.341	0.083	0.078	0.127	0.289
207	0.077	0.323	0.045				

S0004	**SAL**	**LAW**	**DUR**	**Nam**	**Moz**	**Kol**	**KK**
158							0.056
160		0.065		0.208	0.044		0.278
162							0.056
164			0.091	0.250	0.038		0.028
166	0.740	0.226	0.386	0.333	0.472	0.328	0.250
168							0.111
170	0.180	0.548	0.386	0.188	0.081	0.086	0.139
172	0.020	0.113	0.136		0.291	0.552	
174	0.060	0.048		0.021	0.069	0.034	0.028
176					0.006		0.056

S0165	**SAL**	**LAW**	**DUR**	**Nam**	**Moz**	**Kol**	**KK**
136					0.015	0.033	
138	0.269	0.533	0.545		0.066	0.017	
142	0.077	0.033		0.688	0.099	0.017	0.200
144					0.057	0.192	0.175
146	0.462	0.033	0.182		0.256	0.008	0.300
148	0.154	0.233		0.063	0.422	0.633	0.050
150					**0.006**		
152					**0.003**		
158					**0.009**		
160					**0.018**		
162		0.083		0.104	0.015		0.075
164	0.019	0.067	0.114	0.146	0.021	0.025	0.200
166	0.019	0.017	0.068		0.009	0.075	
170			**0.091**				
172					**0.003**		

S0217	**SAL**	**LAW**	**DUR**	**Nam**	**Moz**	**Kol**	**KK**
143			0.159		0.006		0.125
145	0.385	0.403	0.409	0.333	0.223	0.093	0.225
147							**0.275**
149				0.271	0.084	0.017	0.050
155		0.016		0.396	0.352	0.390	

156					**0.003**		
157	0.019		0.091			0.305	
159					0.196	0.008	
161	0.365	0.548	0.318		0.006	0.161	0.100
163	0.019	0.016			0.009		
165	0.212	0.016	0.023		0.117	0.025	0.225
167					**0.003**		

SW225	SAL	LAW	DUR	Nam	Moz	Kol	KK
91	0.115				0.003		
93	0.192	0.161	0.136	0.021	0.170	0.033	0.095
95	0.154	0.145	0.386				0.119
96					**0.003**		
97			**0.068**				
99							**0.071**
101					0.006	0.180	
103	0.019		0.045	0.250	0.194	0.066	0.048
105	0.019	0.016	0.045	0.250	0.161	0.230	0.429
107	0.058	0.097	0.045	0.083	0.015	0.189	0.119
109	0.077	0.323	0.068	0.229	0.048	0.139	0.048
111	0.058	0.177	0.136		0.009	0.057	0.024
113		0.016	0.068		0.133	0.016	
115	0.077	0.016		0.167	0.155	0.066	0.048
117	0.173	0.048			0.003		
119	0.058				0.042	0.016	
121					0.058	0.008	

SW1041	SAL	LAW	DUR	Nam	Moz	Kol	KK
94	0.385	0.581	0.568	0.417	0.419	0.133	0.632
96						**0.075**	
98	0.058	0.081	0.023		0.130		0.316
100						0.150	0.053
102	0.558	0.339	0.409	0.583	0.449	0.642	
104					**0.003**		

SW21	SAL	LAW	DUR	Nam	Moz	Kol	KK
126				0.042	0.042	0.186	
128	0.519	0.565	0.705	0.667	0.515	0.169	
130	0.038		0.023	0.042	0.012	0.076	

132		0.097	0.023	0.188	0.027		
136	0.058	0.081	0.045		0.003	0.356	
138	0.385	0.258	0.182	0.042	0.316	0.195	0.026
140					0.006	0.008	
142							**0.237**
144			0.023				0.184
146						0.008	0.421
148				0.021	0.003		0.132
156					**0.075**		

SW2404	SAL	LAW	DUR	Nam	Moz	Kol	KK
160					0.003	0.059	
162	0.173	0.194			0.015		0.028
164	0.058	0.065		0.563	0.082	0.212	0.139
166	0.385	0.452	0.455	0.188	0.192	0.322	0.389
168	0.269	0.274	0.136	0.250	0.168	0.195	0.278
170							**0.028**
172	0.019		0.250		0.018	0.008	0.028
174					0.006	0.059	
176	0.077	0.016	0.159		0.113	0.093	0.056
178	0.019				0.241	0.042	0.056
180					0.162	0.008	

S0035	SAL	LAW	DUR	Nam	Moz	Kol	KK
162					**0.003**		
164					**0.015**		
166					**0.018**		
172	0.019				0.099		
174					**0.009**		
176	0.019	0.048	0.523	0.063	0.036	0.331	0.050
178	0.519	0.435		0.438	0.660	0.593	0.100
180	0.212	0.145	0.477	0.208	0.096	0.025	0.600
182	0.231	0.323		0.292	0.063	0.051	
184		0.048					0.050
186							**0.050**
190							**0.050**
192							**0.100**

S0006	SAL	LAW	DUR	Nam	Moz	Kol	KK
218	0.308	0.267		0.042	0.130	0.314	
220	**0.019**						
224	0.058			0.188	0.076		
226				0.042	0.012		
234				0.125	0.076		
236			**0.068**				
238	0.192	0.150		0.042	0.027	0.119	
240	0.038	0.217	0.045	0.021	0.012	0.017	
242	0.038	0.133	0.045	0.188	0.03		
244	0.288	0.233	0.568	0.313	0.427	0.542	0.643
246	0.038		0.273	0.042	0.124		0.357
248					0.085	0.008	
250	**0.019**						

SW749	SAL	LAW	DUR	Nam	Moz	Kol	KK
106	0.923	0.339	0.977	0.729	0.696	0.708	0.25
108		0.032			0.042		0.417
110							**0.167**
112	0.077	0.629	0.023	0.271	0.256	0.292	0.083
116					0.006		0.083

SW2410	SAL	LAW	DUR	Nam	Moz	Kol	KK
102							**0.156**
104							**0.094**
106				0.021	0.003		0.031
108	0.942	0.806	0.500	0.396	0.539	0.592	0.469
110					0.006		
112				0.021	0.006		0.094
114							**0.063**
116							**0.031**
118	0.019	0.032	0.205			0.200	
122	0.019	0.129	0.295	0.563	0.114	0.183	0.031
124	0.019				0.139		0.031
126					0.187	0.008	
128					0.006		
132		0.032					
136						0.017	

SW940	SAL	LAW	DUR	Nam	Moz	Kol	KK
138							**0.033**
144	**0.021**						
146				0.042	0.031	0.054	0.033
148	0.396	0.758	0.250	0.021	0.003		
150	0.208	0.032	0.068	0.667	0.146	0.457	0.300
152	0.271	0.048	0.682		0.061	0.250	0.033
154	0.063	0.161		0.021	0.412		0.367
156				0.250	0.337	0.109	0.033
158	0.021						0.033
160					**0.007**		
162					0.003		0.133
164	0.021						0.033
166						**0.109**	
168						**0.022**	

S0295	SAL	LAW	DUR	Nam	Moz	Kol	KK
216							**0.077**
218							**0.038**
220					0.003		0.192
224					0.006		0.038
226		0.032					0.077
228	0.096	0.097		0.021	0.085	0.283	0.038
230	0.404	0.194	0.068	0.063	0.145	0.242	0.231
232	0.385	0.226	0.364	0.125	0.136	0.042	
234	0.058	0.145	0.341	0.479	0.261	0.008	
236	0.058	0.016	0.227				0.038
238							**0.077**
240					0.242	0.350	0.038
242							**0.038**
244							**0.038**
246				0.271	0.039		0.077
252		0.016			0.012	0.008	
256		0.258		0.042	0.006		
259		**0.016**					
264					0.039	0.017	
266					**0.024**		
270						**0.050**	

SW839	SAL	LAW	DUR	Nam	Moz	Kol	KK
147	0.038	0.113	0.795				
149	0.635	0.677	0.205	0.583	0.268	0.267	0.222
155				0.250	0.036	0.025	
157		0.032		0.021	0.319	0.092	0.056
161	0.038				0.003		
165							**0.056**
167	0.019	0.177		0.146	0.021		0.111
169					0.307	0.117	
171	0.058				0.036	0.492	
173	0.212				0.009	0.008	0.222
175							**0.222**
176							**0.111**

SW2406	SAL	LAW	DUR	Nan	Moz	Kol	KK
216					**0.006**		
222					0.052	0.492	
224	0.808	0.403	0.955	0.208	0.176		**1.000**
228					0.088	0.15	
230	0.058	0.097	0.023	0.125	0.282		
236	0.135	0.403	0.023	0.125	0.024	0.025	
238					0.039	0.075	
240				0.146	0.145	0.083	
242					0.006	0.025	
250					0.018	0.008	
252				0.146	0.106	0.008	
254		0.048			0.009	0.008	
256		0.016		0.250	0.045	0.125	
258		0.032			0.003		

SW2419	SAL	LAW	DUR	Nam	Moz	Kol	KK
113					**0.012**		
115		0.016			0.235	0.125	0.050
117		0.032					0.100
119					**0.051**		
121	0.019						0.050
123					0.006		0.250
125	0.192	0.403	0.432	0.042	0.093	0.150	0.300
127	0.154		0.341				0.100

129	0.212	0.048			0.108	0.508	0.050
131	0.096	0.371		0.583	0.199	0.008	
133	0.327	0.129	0.227	0.375	0.054		
135						0.192	0.050
137					0.241	0.017	0.050

SW316	SAL	LAW	DUR	Nam	Moz	Kol	KK
145					**0.025**		
147	0.115	0.161	0.077	0.313	0.108	0.474	0.250
149		0.018	0.154	0.021	0.003	0.009	
151	0.712	0.500	0.077			0.018	
153		0.232	0.308	0.375	0.093	0.237	
155	0.019		0.308		0.049	0.202	
157	0.019				0.071		0.125
159	0.058	0.071			0.102	0.009	
161	0.077	0.018		0.125	0.46	0.026	
163				0.167	0.034	0.026	
165			0.077		0.052		0.250
167							**0.125**
169					0.003		0.250

S0212	SAL	LAW	DUR	Nam	Moz	Kol	KK
230	0.077	0.016		0.021	0.340		0.091
232	0.019	0.113		0.208	0.036	0.069	
234	0.25	0.113	0.136	0.542	0.193	0.431	0.182
236	0.019				0.151	0.069	0.227
238	0.269	0.371	0.432	0.188	0.111	0.207	0.455
240	0.058	0.081	0.114			0.155	
242	0.288	0.306	0.159	0.042	0.084	0.069	
244			**0.159**				
248	**0.019**						
250							**0.045**
260					**0.063**		
262					**0.021**		

SW322	SAL	LAW	DUR	Nam	Moz	Kol	KK
101							**0.025**
103							**0.025**
105	0.038	0.129	0.045		0.006	0.074	0.050

109							**0.025**
111	0.173	0.016		0.042	0.021		0.150
113	0.365	0.452	0.227		0.130	0.139	0.050
115	0.231	0.242	0.159	0.958	0.403	0.393	0.300
117	0.019				0.176		0.100
119	0.058	0.065			0.082	0.320	0.150
121	0.115	0.097	0.568			0.041	0.125
123					0.182	0.033	

S0385	**SAL**	**LAW**	**DUR**	**Nam**	**Moz**	**Kol**	**KK**
149	0.058	0.435	0.250	0.083	0.304	0.059	0.067
153							**0.033**
155							**0.067**
156					**0.006**		
157							**0.100**
159							**0.167**
161							**0.033**
163	0.058	0.016			0.433		0.300
165					0.003		
167		0.032			0.009		0.067
169							**0.033**
171	0.058	0.065	0.227		0.003		
172	**0.019**						
173	0.385	0.177	0.432	0.583	0.163	0.475	0.133
175	0.423	0.274		0.333	0.077	0.424	
177					0.003	0.025	
179			0.091			0.017	

SW2443	**SAL**	**LAW**	**DUR**	**Nam**	**Moz**	**Kol**	**KK**
200							**0.036**
202	0.019	0.355		0.063	0.120	0.033	0.036
204	0.038	0.048			0.163	0.230	0.036
206	0.096	0.306	0.614		0.316	0.131	
208	0.212	0.032			0.048	0.115	0.214
210	0.365	0.097		0.896	0.346	0.418	0.429
212	0.173	0.129	0.386			0.008	
214	0.038						0.250
216	0.058	0.016		0.042	0.006	0.008	
218		0.016				0.057	

APPENDIX II: HARDY-WEINBERG EQUILIBRIUM

A per locus analysis of the expected heterozygosity values deviating from HWE ($P < 0.05$)

SA LANDRACE				
Locus	**#Genot**	**Obs.Het.**	**Exp.Het.**	**P-value**
S0073	26	0.462	0.759	0.000
SW35	26	0.385	0.470	0.233
S0298	26	0.538	0.551	0.576
SW1134	26	0.115	0.180	0.186
SW1851	26	0.577	0.599	0.150
SW2456	26	0.346	0.624	0.003
SW2514	26	0.577	0.743	0.013
SW983	26	0.731	0.486	0.007
S0120	26	0.808	0.760	0.192
SW2	26	0.692	0.765	0.392
SW1557	26	0.538	0.714	0.112
SW378	26	0.346	0.382	0.629
SW761	26	0.385	0.419	0.771
SW2008	26	0.500	0.506	0.055
SW995	26	0.500	0.708	0.060
SW325	26	0.423	0.360	1.000
SW472	26	0.269	0.340	0.285
SW949	26	0.346	0.345	0.645
S0004	25	0.400	0.424	0.389
S0165	26	0.154	0.698	0.000
S0217	26	0.923	0.686	0.016
SW225	26	0.692	0.891	0.018
SW1041	26	0.500	0.548	0.654
SW21	26	0.500	0.589	0.165
SW2404	26	1.000	0.754	0.000
S0035	26	0.500	0.644	0.309
S0006	26	0.808	0.792	0.451
SW749	26	0.154	0.145	1.000
SW2410	26	0.115	0.113	1.000
SW940	24	0.542	0.737	0.080
S0295	26	0.538	0.686	0.179
SW839	26	0.654	0.557	0.808
SW2406	26	0.346	0.333	0.650
SW2419	26	0.731	0.793	0.529
SW316	26	0.346	0.480	0.038
S0212	26	0.769	0.787	0.019
SW322	26	0.769	0.780	0.072
S0385	26	0.654	0.676	0.737
SW2443	26	0.731	0.791	0.432

LARGE WHITE				
Locus	**#Genot**	**Obs.Het.**	**Exp.Het.**	**P-value**
S0073	31	0.452	0.691	0.006
SW35	31	0.452	0.565	0.368

S0298	31	0.645	0.565	0.541
SW1134	31	0.194	0.565	0.000
SW1851	31	0.452	0.597	0.268
SW2456	30	0.300	0.620	0.000
SW2514	31	0.581	0.680	0.008
SW983	31	0.258	0.237	1.000
S0120	31	0.871	0.827	0.919
SW2	31	0.387	0.759	0.000
SW1557	31	0.516	0.642	0.137
SW378	31	0.677	0.648	0.470
SW761	31	0.613	0.551	0.968
SW2008	31	0.774	0.702	0.696
SW995	31	0.677	0.804	0.134
SW325	31	0.516	0.593	0.100
SW472	31	0.387	0.465	0.436
SW949	31	0.806	0.745	0.849
S0004	31	0.613	0.639	0.006
S0165	30	0.200	0.658	0.000
S0217	31	0.839	0.545	0.003
SW225	31	0.613	0.818	0.001
SW1041	31	0.581	0.551	1.000
SW21	31	0.581	0.609	0.801
SW2404	31	0.968	0.690	0.000
S0035	31	0.613	0.692	0.004
S0006	30	0.933	0.801	0.649
SW749	31	0.484	0.497	0.857
SW2410	31	0.290	0.336	0.326
SW940	31	0.323	0.402	0.221
S0295	31	0.839	0.826	0.299
SW839	31	0.387	0.504	0.157
SW2406	31	0.516	0.673	0.393
SW2419	31	0.806	0.691	0.651
SW316	28	0.607	0.677	0.044
S0212	31	0.645	0.748	0.040
SW322	31	0.774	0.719	1.000
S0385	31	0.774	0.710	0.586
SW2443	31	0.839	0.763	0.491

DUROC

Locus	#Genot	Obs.Het.	Exp.Het.	P-value
S0073	22	0.818	0.660	0.345
SW35	22	0.091	0.090	1.000
S0298	22	0.182	0.169	1.000
SW1134	22	0.091	0.089	1.000
SW1851	22	0.682	0.728	0.682
SW2456	22	0.273	0.724	0.000
SW2514	22	0.682	0.615	0.569
SW983	22	0.364	0.304	1.000
S0120	22	0.636	0.541	1.000
SW2	22	0.455	0.457	0.514

SW1557	22	0.591	0.661	0.071
SW378	22	0.318	0.384	0.569
SW761	22	0.636	0.707	0.571
SW2008	22	0.455	0.504	0.186
SW995	22	0.136	0.132	1.000
SW325	22	0.682	0.710	0.681
SW472	22	0.455	0.507	0.686
SW949	22	0.773	0.709	0.650
S0004	22	0.818	0.690	0.011
S0165	22	0.545	0.659	0.002
S0217	22	0.773	0.714	0.095
SW225	22	0.864	0.812	0.024
SW1041	22	0.455	0.521	0.491
SW21	22	0.545	0.478	0.813
SW2404	22	0.727	0.703	0.103
S0035	22	0.591	0.511	0.666
S0006	22	0.773	0.608	0.037
SW749	22	0.045	0.045	1.000
SW2410	22	0.545	0.635	0.736
SW940	22	0.409	0.479	0.555
S0295	22	0.500	0.711	0.014
SW839	22	0.318	0.333	1.000
SW2406	22	0.091	0.090	1.000
SW2419	22	0.682	0.661	0.152
SW316	13	0.000	0.800	0.000
S0212	22	0.773	0.748	0.959
SW322	22	0.727	0.612	0.803
S0385	22	0.727	0.707	0.941
SW2443	22	0.409	0.485	0.649

		NAMIBIA		
Locus	**#Genot**	**Obs.Het.**	**Exp.Het.**	**P-value**
S0073	24	0.708	0.670	0.865
SW35	24	0.583	0.479	0.409
S0298	24	0.792	0.598	0.189
SW1134	24	0.042	0.042	1.000
SW1851	24	0.583	0.675	0.628
SW2456	24	0.292	0.345	0.111
SW2514	24	0.458	0.685	0.025
SW983				
S0120	24	0.167	0.160	1.000
SW2	24	0.750	0.753	0.157
SW1557	24	0.625	0.672	0.412
SW378	24	0.542	0.488	0.682
SW761	24	0.500	0.451	0.812
SW2008	23	0.565	0.480	0.875
SW995	24	0.583	0.733	0.000
SW325	24	0.125	0.160	0.124
SW472	24	0.458	0.503	0.696
SW949	24	0.625	0.659	0.721

S0004	24	0.792	0.763	0.685
S0165	24	0.167	0.502	0.000
S0217	24	0.667	0.673	0.773
SW225	24	0.833	0.804	0.826
SW1041	24	0.417	0.496	0.671
SW21	24	0.500	0.526	0.312
SW2404	24	0.625	0.598	0.050
S0035	24	0.500	0.691	0.069
S0006	24	0.875	0.826	0.012
SW749	24	0.458	0.403	0.637
SW2410	24	0.583	0.537	0.587
SW940	24	0.375	0.501	0.237
S0295	24	0.667	0.690	0.133
SW839	24	0.583	0.588	0.459
SW2406	24	0.917	0.838	0.333
SW2419	24	0.500	0.528	0.704
SW316	24	0.750	0.733	0.169
S0212	24	0.583	0.639	0.597
SW322	24	0.083	0.082	1.000
S0385	24	0.708	0.553	0.379
SW2443	24	0.208	0.196	1.000

MOZAMBIQUE

Locus	#Genot	Obs.Het.	Exp.Het.	P-value
S0073	165	0.539	0.742	0.000
SW35	166	0.548	0.698	0.000
S0298	165	0.679	0.613	0.000
SW1134	166	0.398	0.674	0.000
SW1851	166	0.602	0.744	0.000
SW2456	166	0.295	0.584	0.000
SW2514	166	0.777	0.844	0.000
SW983	166	0.072	0.082	0.135
S0120	165	0.600	0.711	0.000
SW2	166	0.813	0.749	0.000
SW1557	166	0.771	0.756	0.390
SW378	166	0.578	0.497	0.001
SW761	166	0.741	0.704	0.026
SW2008	161	0.547	0.517	0.589
SW995	166	0.681	0.740	0.000
SW325	166	0.422	0.717	0.000
SW472	165	0.230	0.569	0.000
SW949	166	0.861	0.881	0.000
S0004	160	0.719	0.680	0.000
S0165	166	0.367	0.740	0.000
S0217	166	0.717	0.769	0.000
SW225	165	0.891	0.861	0.104
SW1041	166	0.602	0.608	0.700
SW21	166	0.627	0.628	0.000
SW2404	164	0.805	0.833	0.000
S0035	166	0.410	0.541	0.000

S0006	165	0.818	0.767	0.000
SW749	166	0.392	0.450	0.000
SW2410	166	0.584	0.644	0.000
SW940	147	0.224	0.693	0.000
S0295	165	0.764	0.825	0.000
SW839	166	0.602	0.731	0.000
SW2406	165	0.800	0.845	0.000
SW2419	166	0.813	0.823	0.000
SW316	162	0.537	0.748	0.000
S0212	166	0.795	0.801	0.001
SW322	165	0.782	0.752	0.000
S0385	163	0.632	0.690	0.000
SW2443	166	0.717	0.739	0.061

KOLBROEK

Locus	#Genot	Obs.Het.	Exp.Het.	P-value
S0073	61	0.590	0.743	0.015
SW35	61	0.754	0.709	0.001
S0298	61	0.213	0.225	0.195
SW1134	61	0.672	0.806	0.003
SW1851	61	0.475	0.688	0.000
SW2456	61	0.541	0.649	0.066
SW2514	54	0.296	0.690	0.000
SW983	61	0.475	0.564	0.000
S0120	59	0.492	0.509	0.293
SW2	59	0.475	0.720	0.000
SW1557	59	0.525	0.759	0.000
SW378	60	0.300	0.339	0.018
SW761	58	0.259	0.255	1.000
SW2008	59	0.458	0.635	0.000
SW995	59	0.559	0.748	0.000
SW325	61	0.541	0.552	0.463
SW472	61	0.197	0.357	0.001
SW949	59	0.525	0.820	0.000
S0004	58	0.517	0.585	0.012
S0165	60	0.300	0.559	0.000
S0217	59	0.898	0.725	0.001
SW225	61	0.754	0.853	0.000
SW1041	60	0.483	0.547	0.146
SW21	59	0.763	0.772	0.198
SW2404	59	0.966	0.803	0.000
S0035	59	0.525	0.540	0.030
S0006	59	0.475	0.598	0.036
SW749	60	0.383	0.417	0.542
SW2410	60	0.617	0.581	0.928
SW940	46	0.326	0.710	0.000
S0295	60	0.750	0.740	0.814
SW839	60	0.550	0.670	0.033
SW2406	60	0.550	0.712	0.000
SW2419	60	0.733	0.672	0.526

SW316	57	0.684	0.683	0.090
S0212	58	0.466	0.739	0.000
SW322	61	0.689	0.721	0.216
S0385	59	0.542	0.596	0.688
SW2443	61	0.607	0.744	0.000

		KUNE-KUNE		
Locus	**#Genot**	**Obs.Het.**	**Exp.Het.**	**P-value**
S0073	18	0.389	0.414	0.499
SW35	18	0.833	0.713	0.541
S0298	17	0.353	0.299	1.000
SW1134	17	0.412	0.558	0.092
SW1851	18	0.556	0.625	0.624
SW2456	16	0.438	0.498	0.234
SW2514	18	0.944	0.841	0.051
SW983	20	0.050	0.145	0.022
S0120	21	0.619	0.807	0.021
SW2	21	0.714	0.751	0.361
SW1557	19	0.579	0.622	0.168
SW378	20	0.150	0.142	1.000
SW761	19	0.632	0.717	0.735
SW2008	21	0.476	0.449	1.000
SW995	20	0.650	0.824	0.001
SW325	4	0.500	0.750	0.657
SW472	11	0.091	0.775	0.000
SW949	19	0.526	0.852	0.000
S0004	18	0.556	0.841	0.001
S0165	20	0.850	0.812	0.513
S0217	20	1.000	0.815	0.048
SW225	21	0.714	0.785	0.080
SW1041	19	0.421	0.512	0.048
SW21	19	0.632	0.734	0.079
SW2404	18	0.944	0.765	0.020
S0035	10	0.300	0.642	0.004
S0006	7	0.429	0.495	1.000
SW749	12	0.167	0.754	0.000
SW2410	16	0.313	0.754	0.000
SW940	15	0.467	0.777	0.000
S0295	13	0.538	0.911	0.000
SW839	9	0.333	0.869	0.001
SW2406				
SW2419	10	0.500	0.858	0.001
SW316	4	0.500	0.893	0.121
S0212	11	0.273	0.732	0.000
SW322	20	0.800	0.854	0.214
S0385	15	0.667	0.867	0.043
SW2443	14	0.500	0.730	0.007